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UNITED STATES PATENT APPLICATION

FOR

COMPOSITIONS AND METHODS FOR INHIBITING SQUAMOUS CELL CARCINOMA

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COMPOSITIONS AND METHODS FOR INHIBITING SQUAMOUS CELL CARCINOMA

FIELD

[001] The present invention relates to compositions and methods for detecting and inhibiting squamous cell carcinoma using agents that target the laminin 5 alpha 3 G4-G5 domain.

INTRODUCTION

[002] Squamous cell carcinoma (SCC) is common form of cancer and is the second most common form of skin cancer in the United States. SCCs are highly invasive and metastatic. SCCs are associated with a comparatively high risk of recurrence, resulting in significant mortality. SCC can be diagnosed by biopsy. However, SCCs are typically not as distinct as basal cell carcinomas or melanomas, making detection and diagnosis difficult. Current methods of treatment, i.e. surgery, radiotherapy, and chemotherapy, require continued monitoring due to the metastatic nature of the disease. The development of alternative methods of detection and treatment is therefore desirable.

[003] The compositions and methods described herein are directed towards identifying agents that can detect and inhibit proteins associated with SCC tumorigenesis. Of particular interest, are agents that interact with the laminin-5 alpha 3 chain G4 and/or G5 domains.

SUMMARY

[004] Provided herein are compositions and methods useful for detecting and treating squamous cell carcinoma (SCC). The compositions generally comprise antibodies capable of binding a migration facilitating protein (MFP) of a laminin 5 alpha 3 chain G4 and/or G5 domain or subdomain. MFPs typically comprise 8, 9, 10 or more amino acids present in the laminin 5 alpha 3 G4 and/or 5 domains that do not comprise a recognized cleavage site for bone morphogenetic protein-1 (BMP-1) and BMP-1 related proteins. For example, MFPs can be generated comprising: (1) the G5 subdomain; (2) the G4 subdomain lying between amino acid 1358 and amino acid 1366; (3) the G4 subdomain lying between amino acid

1375 and amino acid 1390; (4) the G4 subdomain lying between amino acid 1399 and 1530; and, (5) the G4-5 subdomain lying between amino acid 1399 and amino acid 1713. As will be appreciated by a person of skill in the art, MFPs encoding other subdomains within the laminin 5 alpha 3 G4 and/or 5 domains can also be generated and used in the methods of the present invention. The compositions can include additional components, such as, detectable labels and a pharmaceutically acceptable carrier.

[005] The methods generally involve administering a therapeutically effective amount of a composition comprising one or more antibodies capable of inhibiting SCC tumorigenesis to a patient diagnosed with SCC. Treatment of a patient diagnosed with SCC with the compositions described herein can be combined with other medical means for treating SCC, such as surgery, radiotherapy, and chemotherapy. The SCC can be selected from the group consisting of skin cancer, lung cancer, head cancer, gastric cancer, colorectal cancer, throat cancer, cancer of the urinary tract, cancer of the reproductive tract, esophageal cancer, and bronchiogenic carcinoma.

[006] Also provided are methods that utilize the MFPs described above. In some embodiments, a method is provided for detecting the binding activity of a candidate agent in a sample that comprises the steps of:

(a) contacting the sample with an MFP under conditions effective to permit binding between the MFP and the candidate agent (if present); and,

(b) detecting the binding of the candidate agent.

[007] A number of different assays can be used to detect binding of the candidate agent. For example, in some embodiments, the candidate agent is labeled and binding determined directly. In other embodiments, the binding of the candidate agent is determined through the use of competitive binding assays in which the competitor is a binding moiety known to bind the MFP, i.e., an antibody. Displacement of the competitor by the candidate agent is an indication that the candidate agent is capable of binding the MFP.

[008] Also provided herein are methods for screening for candidate agents that inhibit SCC tumorigenesis. In some embodiments, a method is provided for screening for candidate agents that inhibit SCC tumor development comprising the steps of:

a) subcutaneously injecting nude mice with a suspension comprising:

i) Ras/IKB transformed epithelial cells;

ii) a migration facilitating protein (MFP) of a laminin G4 and/or G5 domain or subdomain;

iii) one or more candidate agents; and

b) determining the presence or absence of a tumor.

[009] In some embodiments, a method is provided to evaluate the effect of a candidate SCC drug comprising administering the drug to a patient diagnosed with SCC and removing a cell sample from the patient. A number of different assays can be used to evaluate the effect of the candidate drug. For example, the expression profile of the cell sample can be determined and compared with an expression profile of a healthy individual. In some embodiments, the cell sample can be analyzed for the presence or absence of an MFP associated with SCC development before and after treatment with a candidate drug. In yet other embodiments, the size of the tumor before and after treatment with a candidate drug can be analyzed to determine if the drug is effective in inhibiting the invasion of nearby normal cells.

[010] Also provided herein is a method for diagnosing SCC comprising removing a cell sample from an individual and analyzing the cell with one or more MFPs determined to be involved in SCC proliferation and/or metastasis.

[011] These and other features of the compositions and methods described herein will become more apparent from the detailed description below.

BRIEF DESCRIPTION OF THE DRAWINGS

[012] Aspects of the invention can be more fully understood with respect to the following drawings. In the drawings, similar elements are referenced with like numbers.

[013] FIG. 1A provides a cartoon illustrating the G4 (**1**) and G5 (**2**) domains of the alpha 3 chain of laminin 5, including the cleavage recognition sites (**1a**, **1b**, **1c**) for bone morphogenetic protein-1 (BMP-1);

[014] FIG. 1B illustrates exemplary embodiments of migration facilitating proteins (MFPs) that can be generated from the G4 domain;

[015] FIG. 1C illustrates exemplary embodiments of migration facilitating proteins (MFPs) that can be generated from the G5 domain;

[016] FIG. 1D illustrates exemplary embodiments of migration facilitating proteins (MFPs) that can be generated comprising amino acids present in both the G4 and G5 domains;

[017] FIG. 2A illustrates the polynucleotide sequence for *Homo sapiens* laminin-related protein (LAMA3; GenBank accession number L34156; SEQ ID. NO.: 1);

[018] FIG. 2B illustrates the amino acid sequence for *Homo sapiens* laminin-related protein (LAMA3; GenBank accession number L34156; SEQ ID. NO.: 2);

[019] FIG. 2C illustrates the polynucleotide sequence for *Homo sapiens* laminin, alpha 3 transcript variant 1 (LAMA3; GenBank NM_198129; SEQ ID. NO.: 3);

[020] FIG. 2D illustrates the amino acid sequence for *Homo sapiens* laminin, alpha 3 transcript variant 1 (LAMA3; GenBank NM_198129; SEQ ID. NO.: 4);

[021] FIG. 2E illustrates the polynucleotide sequence for *Homo sapiens* laminin, alpha 3 transcript variant 2 (LAMA3; GenBank NM_000227 SEQ ID. NO.: 5);

[022] FIG. 2F illustrates the amino acid sequence for *Homo sapiens* laminin, alpha 3 transcript variant 2 (LAMA3; GenBank NM_000227 SEQ ID. NO.: 6);

[023] FIG. 2G illustrates the polynucleotide sequence for *Rattus norvegicus* laminin 5 alpha 3 (LAMA3; GenBank NM_173306; SEQ ID. NO.: 7);

[024] FIG. 2H illustrates the amino acid sequence for *Rattus norvegicus* laminin 5 alpha 3 (LAMA3; GenBank NM_173306; SEQ ID. NO.: 8);

[025] FIG. 2I illustrates the polynucleotide sequence for *Mus musculus* laminin, alpha 3 (LAMA3; GenBank XM 140451; SEQ ID. NO.: 9);

[026] FIG. 2J illustrates the amino acid sequence for *Mus musculus* laminin, alpha 3 (LAMA3; GenBank XM 140451; SEQ ID. NO.: 10);

[027] FIG. 3A illustrates an exemplary *Homo sapiens* amino acid sequence for the combined G4 and G5 domains (SEQ ID NO.: 11);

[028] FIG. 3B illustrates an exemplary *Homo sapiens* polynucleotide sequence for the combined G4 and G5 domains (SEQ ID NO.: 12);

[029] FIG. 3C illustrates an exemplary *Homo sapiens* amino acid sequence for the G5 domain (SEQ ID NO.: 13);

[030] FIG. 3D illustrates an exemplary *Homo sapiens* polynucleotide sequence for the G5 domain (SEQ ID NO.: 14);

[031] FIG. 3E illustrates an exemplary *Homo sapiens* amino acid sequence for the G4 subdomain delimited by amino acid 1356 through amino acid 1366 (SEQ ID NO.: 15);

[032] FIG. 3F illustrates an exemplary *Homo sapiens* polynucleotide sequence for the G4 subdomain delimited by nucleotide 4067 through nucleotide 4099 (SEQ ID NO.: 16);

[033] FIG. 3G illustrates an exemplary *Homo sapiens* amino acid sequence for the G4 subdomain delimited by amino acid 1375 through amino acid 1390 (SEQ ID NO.: 17);

[034] FIG. 3H illustrates an exemplary *Homo sapiens* polynucleotide sequence for the G4 subdomain delimited by nucleotide 4124 through nucleotide 4171 (SEQ ID NO.: 18);

[035] FIG. 3I illustrates an exemplary *Homo sapiens* amino acid sequence for the G4 subdomain delimited by amino acid 1399 through amino acid 1529 (SEQ ID NO.: 19);

[036] FIG. 3J illustrates an exemplary *Homo sapiens* polynucleotide sequence for the G4 subdomain delimited by nucleotide 4196 through nucleotide 4588 (SEQ ID NO.: 20);

[037] FIG. 3K illustrates an exemplary *Homo sapiens* amino acid sequence for a polypeptide spanning the G4 and G5 domain delimited by amino acid 1399 through amino acid 1713 (SEQ ID NO.: 21);

[038] FIG. 3L illustrates an exemplary *Homo sapiens* polynucleotide sequence for a polynucleotide spanning the G4 and G5 domain delimited by nucleotide 4196 through nucleotide 5140 (SEQ ID NO.: 22);

[039] FIG. 3M illustrates an exemplary *Homo sapiens* amino acid sequence for a polypeptide spanning the G5 domain delimited by amino acid 1560 through amino acid 1713 (SEQ ID NO.: 24);

[040] FIG. 3M illustrates an exemplary *Homo sapiens* polynucleotide sequence for a polynucleotide spanning the G5 domain delimited by nucleotide 4679 through nucleotide 5140 (SEQ ID NO.: 26);

[041] FIG. 4 depicts normal human skin epithelia cells and SCC epithelial derived tumor cells;

[042] FIG. 5 illustrates the results from a migration assay comparing wild-type cells, and keratinocytes transformed with truncated versions of the laminin-5 alpha 3 chain;

[043] FIG. 6 illustrates a scratch assay in which cells lacking the G4 and G5 domain (i.e. 1337TR) migrate more efficiently than cells expressing wild-type laminin-5 alpha 3 chain;

[044] FIG. 7 illustrates a mouse model of human SCC. The left panel depicts tumor formation in nude mice transformed with RAS/IKB keratinocytes transformed with wild-type laminin-5 alpha 3 chain. The middle and right panels illustrate that SCC tumors are not formed in nude mice transformed with RAS/IKB laminin-5 negative keratinocytes (right panel) or with a laminin-5 construct lacking the G4 and G5 domains (middle panel).

DETAILED DESCRIPTION

[045] It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of the inventions described herein. In this application, the use of the singular includes the plural unless specifically state otherwise. Also, the use of "or" means "and/or" unless state otherwise. Similarly, "comprise," "comprises," "comprising," "include," "includes" and "including" are not intended to be limiting. Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art to which the invention belongs.

1. Definitions

[046] As used herein, the following terms and phrases are intended to have the following meanings:

[047] "Antibody" has its standard meaning and is intended to refer to intact molecules as to fragments thereof, such as Fab, F(ab')2, and Fv fragments, that are capable of binding an epitope.

[048] "Cancer" has its standard meaning and is intended to refer to any malignant tumor of potentially unlimited growth that expands locally by proliferation and systemically by metastasis.

[049] "Neoplasm" has its standard meaning and is intended to refer to the abnormal growth of a tissue, such as a tumor.

[050] "Nucleobase" means those naturally occurring and those synthetic nitrogenous, aromatic moieties commonly found in the nucleic acid arts. Examples of nucleobases include purines and pyrimidines, genetically encoding nucleobases, analogs of genetically encoding nucleobases, and purely synthetic nucleobases. Specific examples of genetically encoding nucleobases include adenine, cytosine, guanine, thymine, and uracil. Specific examples of analogs of genetically encoding nucleobases and synthetic nucleobases include 5-methylcytosine, pseudoisocytosine, 2-thiouracil and 2-thiothymine, 2-aminopurine, N9-(2-amino-6-chloropurine), N9-(2,6-diaminopurine), hypoxanthine, N9-(7-deaza-guanine), N9-(7-deaza-8-aza-guanine) and N8-(7-deaza-8-aza-adenine). 5-propynyl-uracil, 2-thio-5-propynyl-uracil. Other non-limiting examples of suitable nucleobases include those nucleobases illustrated in Figures 2(A) and 2(B) of U.S. Patent 6,357,163, incorporated herein by reference in its entirety.

[051] "Nucleoside" refers to a nucleobase linked to a pentose sugar. Pentose sugars include ribose, 2'-deoxyribose, 3'-deoxyribose, and 2', 3'-dideoxyribose.

[052] "Nucleoside analog" refers to a nucleobase linked to a sugar, other than a pentose sugar. For example, a nucleobase linked to hexose.

[053] "Nucleotide" refers to compound comprising a nucleobase, a pentose sugar and a phosphate. Thus, as used herein a nucleotide refers to a phosphate ester of a nucleoside, e.g., a triphosphate.

[054] "Nucleobase Polymer or Oligomer" refers to two or more nucleobases that are connected by linkages that permit the resultant nucleobase polymer or oligomer to hybridize to a polynucleotide having at least a partially complementary nucleobase sequence. Nucleobase polymers or oligomers include, but are not limited to, poly- and oligonucleotides (e.g., DNA and RNA polymers and oligomers), poly- and oligonucleotide analogs and poly- and oligonucleotide mimics, such as polyamide nucleic acids or peptide

nucleic acids. Polyamide nucleic acids and peptide nucleic acids are two different phrases used in the literature to describe the same molecule, abbreviated herein as PNA. Nucleobase polymers or oligomers can vary in size from a few nucleobases, for example, from 2 to 40 nucleobases, to several hundred nucleobases, to several thousand nucleobases, or more.

[055] "Polynucleotides or Oligonucleotides" refer to nucleobase polymers or oligomers in which the nucleobases are linked by sugar phosphate linkages (sugar-phosphate backbone). Exemplary poly- and oligonucleotides include polymers of 2'-deoxyribonucleotides (DNA) and polymers of ribonucleotides (RNA). A polynucleotide may be composed entirely of ribonucleotides, entirely of 2'-deoxyribonucleotides or combinations thereof.

[056] "Polynucleotide or Oligonucleotide Analog" refers to nucleobase polymers or oligomers in which the nucleobases are linked by a phosphate backbone comprising one or more sugar analogs or phosphate analogs. Typical oligonucleotide or polynucleotide analogs include, but are not limited to, sugar alkylphosphonates, sugar phosphoramidites, sugar alkyl- or substituted alkylphosphotriesters, sugar phosphorothioates, sugar phosphorodithioates, sugar phosphates and sugar phosphate analogs in which the sugar is other than 2'-deoxyribose or ribose, nucleobase polymers having positively charged sugar-guanidyl interlinkages such as those described in U.S. Patent No. 6,013,785 and U.S. Patent No. 5,696,253 (see also, Dagani 1995, Chem. & Eng. News 4-5:1153; Dempey et al., 1995, J. Am. Chem. Soc. 117:6140-6141). Such positively charged analogues in which the sugar is 2'-deoxyribose are referred to as "DNGs," whereas those in which the sugar is ribose are referred to as "RNGs." Specifically included within the definition of poly- and oligonucleotide analogs are locked nucleic acids (LNAs; see, e.g. Elayadi et al., 2002, Biochemistry 41:9973-9981; Koshkin et al., 1998, J. Am. Chem. Soc. 120:13252-3; Koshkin et al., 1998, Tetrahedron Letters, 39:4381-4384; Jumar et al., 1998, Bioorganic & Medicinal Chemistry Letters 8:2219-2222; Singh and Wengel, 1998, Chem. Commun.,

12:1247-1248; WO 00/56746; WO 02/28875; and, WO 01/48190; all of which are incorporated herein by reference in their entireties).

[057] "Polynucleotide or oligonucleotide mimic" refers to nucleobase polymers or oligomers in which the nucleobases are connected by a linkage other than a sugar-phosphate linkage or a sugar-phosphate analog linkage. Mimics with a specific linkage include peptide nucleic acids (PNAs) as described in any one or more of United States Patent Nos. 5,539,082, 5,527,675, 5,623,049, 5,714,331, 5,718,262, 5,736,336, 5,773,571, 5,766,855, 5,786,461, 5,837,459, 5,891,625, 5,972,610, 5,986,053, 6,107,470, 6,451,968, 6,441,130, 6,414,112 and 6,403,763; all of which are incorporated herein by reference. Other types of mimics are described in the following publications: Lagriffoul et al., 1994, *Bioorganic & Medicinal Chemistry Letters,* 4: 1081-1082; Petersen et al., 1996, *Bioorganic & Medicinal Chemistry Letters, 6:* 793-796; Diderichsen et al, 1996, *Tett. Lett. 37:* 475-478; Fujii et al., 1997, *Bioorg. Med. Chem. Lett. 7:* 637-627; Jordan et al., 1997, *Bioorg. Med. Chem. Lett. 7:* 687-690; Krotz et al., 1995, *Tett. Lett. 36:* 6941-6944; Lagriffoul et al, 1994, *Bioorg. Med. Chem. Lett.* 4: 1081-1082; Diederichsen, U., 1997, *Bioorganic & Medicinal Chemistry* 25 *Letters, 7:* 1743-1746; Lowe et al., 1997, *J. Chem. Soc. Perkin Trans. 1, 1:* 539-546; Lowe et al., 1997, *J.Chem. Soc. Perkin Trans. 11:* 547-554; Lowe et al., 1997, *I. Chem. Soc. Perkin Trans. 1 1:5* 55-560; Howarth et al., 1997, *I. Org. Chem. 62:* 5441-5450; Altmann, K-H et al., 1997, *Bioorganic & Medicinal Chemistry Letters, 7:* 1119-1122; Diederichsen, U., 1998, *Bioorganic & Med. Chem. Lett., 8:*165-168; Diederichsen et al., 1998, *Angew. Chem. mt. Ed., 37:* 302-305; Cantin et al., 1997, *Tett. Lett., 38:* 4211-4214; Ciapetti et al., 1997, *Tetrahedron, 53:* 1167-1176; Lagriffoule et al., 1997, *Chem. Eur. 1.' 3:* 912-919; Kumar et al., 2001, *Organic Letters 3(9):* 1269-1272; and the Peptide-Based Nucleic Acid Mimics (PENAMs) of Shah et al. as disclosed in WO 96/04000. All of which are incorporated herein by reference.

[058] The oligonucleotides may be single stranded or double stranded, as specified, or contain portions of both double stranded or single stranded sequence. As will be appreciated by those in the art, the depiction of a single strand ("Watson") also defines the

sequence of the other strand ("Crick"); thus the sequences described herein also includes the complement of the sequence.

[059] "Protein" has its standard meaning and is intended to refer to at least two covalently attached amino acids, which includes proteins, polypeptides, oligopeptides and peptides. The protein may be made up of naturally occurring amino acids and peptide bonds, or synthetic peptidomimetic structures, i.e., "analogs" such as peptoids [see Simon et al., Proc. Natl. Acad. Sci. U.S.A. 89(20:9367-71 (1992)], generally depending on the method of synthesis. Thus "amino acid", or "peptide residue", as used herein means both naturally occurring and synthetic amino acids. For example, homo-phenylalanine, citrulline, and noreleucine are considered amino acids for the purposes of the invention. "Amino acid" also includes imino acid residues such as proline and hydroxyproline. In addition, any amino acid representing a component of the variant proteins of the present invention can be replaced by the same amino acid but of the opposite chirality. Thus, any amino acid naturally occurring in the L-configuration (which may also be referred to as the R or S, depending upon the structure of the chemical entity) may be replaced with an amino acid of the same chemical structural type, but of the opposite chirality, generally referred to as the D- amino acid but which can additionally be referred to as the R- or the S-, depending upon its composition and chemical configuration. Such derivatives generally have the property of greatly increased stability, and therefore are advantageous in the formulation of compounds which may have longer in vivo half lives, when administered by oral, intravenous, intramuscular, intraperitoneal, topical, rectal, intraocular, or other routes.

[060] In some embodiments, the amino acids are in the (S) or L-configuration. If non-naturally occurring side chains are used, non-amino acid substituents may be used, for example to prevent or retard in vivo degradations. Proteins including non-naturally occurring amino acids may be synthesized or in some cases, made recombinantly; see van Hest et al., FEBS Lett 428:(1-2) 68-70 May 22 1998 and Tang et al., Abstr. Pap Am. Chem. S218: U138 Part 2 August 22, 1999, both of which are expressly incorporated by reference herein.

[061] "Squamous cell carcinoma" has its standard meaning and is intended to refer to any neoplasm or tumor of epithelial cells.

[062] "Tumorigenesis" has its standard meaning and is intended to refer to the basic developmental processes that produce tumors. These basic properties include the ability to proliferate or invade nearby normal cells and the ability to migrate from the site where the tumor initiated, i.e. metastasis.

2. Exemplary Embodiments

[063] Provided herein are: (1) migration facilitating proteins (MFPs) derived from the laminin-5 $\alpha 3$ chain G4 and/or G5 domains; (2) antibodies which bind to MFPs, thereby inhibiting tumorigenesis of neoplastic epithelial cells; (3) methods for screening for agents, such as antibodies, small molecules, etc., that specifically bind one or more of the MFPs described herein; (4) methods for screening for agents that inhibit squamous cell carcinoma (SCC) tumor development using MFPs, (5) methods for diagnosing SCC; and, (6) methods for determining the efficacy of candidate agents used to treat SCC. All of these inventions rely upon MFPs, nucleic acids that encode MFPs and other molecules, such as antibodies, that bind MFPs.

[064] Laminin-5 (formerly called kalinin, nicein, or BM6000) is a heterotrimeric extracellular matrix protein that is initially synthesized and secreted in an unprocessed form with an $\alpha 3$ chain of 200 kDa, a $\beta 3$ chain of 140 kDA, and a $\gamma 2$ chain of 155 kDA. (Marinkovich et al., 1992, *J. Biol. Chem.*, 267: 17900-17906). Laminin-5 is a component of the basal lamina, the structure that provides tissue integrity, as well as the foundation for migration, growth and differentiation of cells. It is therefore not surprising that processes that interfere with wild type functions of laminin-5 produce diseases in humans and other mammals.

[065] Large deposits of laminin-5 are found at the leading edges of squamous cell carcinomas (SCCs). This deposition of laminin-5 is believed to serve as a substrate for tumor invasion (see, e.g., Pyke et al., 1995, *Canc. Res.* 55: 4132-4139; Berndt et al., 1997,

Invasion and Metastasis, 17: 251-258). Increased laminin-5 immunoreactivity is indicative of a poor prognosis in patients with squamous cell carcinoma (SCC). Laminin-5 is also preferentially expressed by invading malignant cells of many human carcinomas in additions to SCCs, colon and mammary carcinomas (Pyke, et al., 1994, *Am. J. Pathol.* 145(4):782-791) and malignant gliomas (Fukushima et al., 1998, *Int. J. Cancer*, 76: 63-72).

[066] Processing of extracellular matrix proteins by proteases is emerging as a key mechanisms in processes such as wound healing and tumor metastasis. Several proteases have been implicated in laminin-5-processing (see, e.g., Veitch et al., 2003, *J. Biol. Chem.*, 278: 15661-15668; and U.S. Pat. Pub. No. 2002/0076736). In fully formed tissues, laminin-5 is completely processed and is devoid of the G4 and G5 domains (Marinkovich et al., 1992, *J. Biol. Chem.*, 267:17900-17906). Without being bound by theory, it appears that specific proteolytic processing can convert laminin-5 from a pro-migratory signal required for cell migration during tumor invasion and tissue remodeling to an adhesive substrate devoid of the G4 and G5 domains.

2.1 Migration Facilitating Sequences

[067] Accordingly, provided herein are polynucleotide and amino acid sequences associated with SCC, herein termed "migration facilitating sequences" or "MFSs". The proteins having the various amino acid sequences are referred to herein as "migration facilitating proteins" or "MFPs". Association in this context means that the amino aicd and polynucleotide sequences are either differentially expressed or altered in SCCs or neoplastic epithelial cells as compared to normal epithelial tissue. "SCC" refers herein to any malignant neoplasm or tumor of epithelial cells. Specific examples of epithelial cells include squamous cells, squamous carcinoma cells, keratinocytes, mucosal epithelial cells, such as oral mucosal cells, gastrointestinal epithelial cells, corneal epithelium of the eye, and epithelial cells of the urinary and reproductive tract. Specific examples of SCC carcinomas arising from neoplastic epithelial cells include skin, lung, head, neck, oral, gastric, colorectal, throat, urinary tract, reproductive tract, esophageal, etc.

[068] SCC is commonly sun-induced, i.e., actinically derived SCC. SCC can also result from transplant or invasive surgery, or follow other immunosuppressive situations. Chronic inflammation can lead to development of SCC at the site of inflammation, e.g., a burn or scar, Marjolin's ulcer, etc. SCC can be virally induced, for example, SCC can result from human papillomavirus-induced (HPV) infection. SCC can include adenoid (acantholytic) SCC, spindle cell SCC, verrucous carcinoma (VC), keratoacanthoma (KA), nodular SCC periungual SCC, and other epithelial carcinomas.

[069] MFSs can include both polynucleotide and amino acid sequences. In some embodiments, the MFSs are recombinant polynucleotides. By the term "recombinant polynucleotide" herein is meant polynucleotides, originally formed *in vitro*, in general, by the manipulation of the polynucleotide by polymerases and endonucleases, in a form not normally found in nature. Thus, an isolated polynucleotide, in a linear form, or an expression vector formed *in vitro* by ligating polynucleotide molecules that are not normally joined, are both considered recombinant for the purposes of this invention. It is understood that once a recombinant polynucleotide is made and reintroduced into a host cell or organism, it will replicate non-recombinantly, *i.e.* using the *in vivo* cellular machinery of the host cell rather than *in vitro* manipulations; however, such polynucleotides, once produced recombinantly, although subsequently replicated non-recombinantly, are still considered recombinant for the purposes of the invention.

[070] As will be appreciated by those in the art, and more fully outlined below, MFSs comprising polynucleotides are useful in a variety of applications, including diagnostic applications, where they can be used as hybridization probes to detect MFSs in SCCs, as well as in therapeutic applications, such as the development of antisense sequences that can be used to affect the expression and activity of MFPs in SCCs.

[071] MFSs include those that are up-regulated, (e.g., expressed at a higher level), as well as those that are down-regulated, (e.g., expressed at a lower level) in SCCs. MFSs also include sequences that have been altered (i.e. truncated sequences or sequences with a one or more mutations, such as point mutations, deletions, insertions, etc.) and show either

the same expression profile or an altered profile. In some embodiments, the MFSs are from humans. However, as will be appreciated by a person of skill in the art, MFSs from other organism may be useful in animal models of disease and drug evaluation. Thus, other MFSs are provided. For example, MFSs can be obtained from vertebrates, including mammals, such as rodents (rats, mice, hamsters, guinea pigs, etc.), primates, farm animals (including sheep, goats, pigs, cows, horses, etc), as well invertebrates, such as *Drosophila*. MFSs from other organisms may be obtained using the techniques outlined below.

[072] In some embodiments, MFSs are those that are altered but show either the same expression profile or an altered profile as compared to normal epithelial tissue of the same differentiation stage. "Altered MFSs" as used herein refers to sequences which are truncated, contain insertions or contain point mutations.

[073] An MFS can be initially identified by substantial nucleic acid and/or amino acid sequence homology to the MFS's outlined herein. Such homology can be based upon the overall oligonucleotide or amino acid sequence, and is generally determined, using either homology programs or hybridization conditions. As is known in the art, a number of different programs are available for determining polynucleotide or amino acid sequence homology including sequence based alignment programs, sequence homology based alignment programs, structural alignment programs etc. Non-limiting examples of sequence-based alignment programs include Smith-Waterman searches (Smith & Waterman, *Adv. Appl. Math.* 2:482 (1981)), Needleman-Wunsch (Needleman & Wunsch, *J. Mol. Biol.* 48:443 (1970)), Double Affine Smith-Waterman, frame search, Gribskov/GCG profile search, Gribskov/GCG profile scan, profile frame search, Bucher generalized profiles, Hidden Markov models, Hframe, Double Frame, Blast, Psi-Blast, Clustal, and GeneWise. Sequence homology based alignment methods are described in Altschul et al. (Altschul et al., *J. Mol. Biol.* 215(3):403 (1990)). Examples of structural alignment programs include VAST from the NCBI; SSAP (Orengo and Taylor, *Methods Enzymol* 266(617-635 (1996)) SARF2 (Alexandrov, *Protein Eng* 9(9):727-732. (1996)) CE (Shindyalov and Bourne, *Protein Eng* 11(9):739-747. (1998)); (Orengo et al., *Structure*

5(8):1093-108 (1997); Dali (Holm et al., *Nucleic Acid Res.* 26(1):316-9 (1998), Computerized implementations of some of the above described algorithms are also available (e.g., BLASTx, BLAST, GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Drive, Madison, WI); the Best Fit sequence program described by Devereux et al., *Nucl. Acid Res.* 12:387-395 (1984).

[074] Polynucleotide homology can also be determined through hybridization studies; see for example Maniatis et al., Molecular Cloning: A Laboratory Manual, 2d Edition, 1989, and Short Protocols in Molecular Biology, ed. Ausubel, et al., both of which are hereby incorporated by reference. Generally, stringent conditions are selected, although less stringent hybridization conditions can be used. Typically, stringent conditions are selected to be about 5-10/C lower than the thermal melting point (Tm) for the specific sequence at a defined ionic strength pH. An extensive guide to the hybridization of nucleic acids is found in Tijssen, Techniques in Biochemistry and Molecular Biology--Hybridization with Nucleic Acid Probes, "Overview of principles of hybridization and the strategy of nucleic acid assays" (1993).

[075] In some embodiments, MFSs are polynucleotides. Polynucleotides comprising MFSs can be generated from either a full length genomic and/or cDNA polynucleotide encoding a laminin-5 α3 chain. In some embodiments, MFSs are generated from the human α3 chain of laminin-5 (Ryan et al., 1994, J. Biol. Chem., 269: 22779-22787; Gen Bank Accession No. NM_000227). MFSs of various lengths spanning the G4 and/or G5 domains can be generated. For example, an polynucleotide spanning a subdomain of the G4 domain of the human α3 chain of laminin-5 can be generated by starting at nucleotide position 4196 and ending at nucleotide 4588. An oligonucleotide spanning the G5 domain of the human α3 chain of laminin-5 can be generated by starting at nucleotide position 4590 and ending at nucleotide 5140. An oligonucleotide spanning a subdomain of the G4 domain and the entire G5 domain of the human α3 chain of laminin-5 can be generated by starting at nucleotide position 4196 and ending at nucleotide 5140.

[076] The exact number of nucleotides or nucleotide analogs chosen will vary depending on the sequence of the nucleotides selected and the presence of nucleotides encoding amino acids that comprise antigenic determinants. By "epitope" or "determinant" "or antigenic determinant" herein is meant a portion of a protein that can generate and/or bind an antibody or T-cell receptor in the context of MHC. For example, the presence of antigenic determinants within the G4 and G5 domains can be identified by searching databases for MHC ligands and peptide motifs (Rammensee, H., et al. (1999) Immunogenetics, 50:213-219). This information can be used to generate MFSs comprising MHC epitopes. Typically, epitopes recognized by MHC class I molecules comprise between 8 and 11 amino acids, thus, an MFS encoding an MHC class I epitope can range between 24 to 33 nucleotides. Viral peptides recognized by MHC class II molecules comprise between 10 to 20 amino acids, thus, an MFS encoding an MHC class II epitope can range between 30 to 60 nucleotides (Fundamental Immunology, 4th edition, W. E. Paul, ed., Lippincott-Raven Publishers, 1999, Chapter 39, pp 1295-1334). In other embodiments, MFSs range between 24 to 1050, or from 60 to 300 nucleotides, or from 60 to 405 nucleotides, or from 60 to 555 nucleotides, or from 60 to 600 nucleotides, or from 60 to 750 nucleotides, or from 60 to 900 nucleotides or from 60 to 1050 nucleotides. In yet other embodiments, MFSs range from 150 to 300 nucleotides, or from 150 to 405 nucleotides, or from 150 to 450 nucleotides, or from 150 to 525 nucleotides, or from 150 to 600 nucleotides, or from 150 to 750 nucleotides, or from 150 to 1050 nucleotides, or from 300 to 600 nucleotides, or from 300 to 900 nucleotides, or from 300 to 1050 nucleotides.

2.2 Migration Facilitating Proteins

[077] In some embodiments, "migration facilitating proteins" or "MFPs" are generated from the amino acid sequence encoding the laminin-5 $\alpha 3$ G4 and/or G5 domains or subdomains thereof. "MFPs" are proteins that are capable of supporting migration of nearby tissue or tissue located at distal points in the body by neoplastic epithelial cells. MFPs also can be recombinant. A "recombinant MFP protein" is a protein made using recombinant techniques, i.e. through the expression of a recombinant oligonucleotide as

described above. A recombinant protein is distinguished from a naturally occurring protein by at least one or more characteristics. For example, the protein may be isolated or purified away from some or all of the proteins and compounds with which it is normally associated in its wild type host, and thus may be substantially pure. Generally, an isolated protein is unaccompanied by at least some of the material with which it is normally associated in its natural state, preferably constituting at least about 0.5%, more preferably at least about 5% by weight of the total protein in a given sample. A substantially pure protein comprises at least about 75% by weight of the total protein, with at least about 80% being preferred, and at least about 90% being particularly preferred. The definition includes the production of an MFP from one organism in a different organism or host cell. Alternatively, the protein may be made at a significantly higher concentration than is normally seen, through the use of an inducible promoter or high expression promoter, such that the protein is made at increased concentration levels. Alternatively, the protein may be in a form not normally found in nature, as in the addition of an epitope tag or amino acid substitutions, insertions and deletions, as discussed below.

[078] In some embodiments, MFPs are generated from the G4 and G5 domains of the human α3 chain of laminin-5 (see FIG. 1A). MFPs of various lengths spanning the G4 and/or G5 domains can be generated. FIG. 1B illustrates an exemplary embodiment of the generation of MFPs from the G4 domain. As illustrated in FIGS. 1B-D, a number of MFPs can be generated from the G4 and/or G5 domains comprising varying numbers of amino acids or amino acid analogs. The exact number of amino acids or amino acid analogs chosen will vary depending on the sequence of the amino acids selected, the presence of bone morphogenetic-1 cleavage sites, and the presence of amino acids comprising antigenic determinants.

[079] As discussed above, the presence of antigenic determinants within the G4 and G5 domains can be identified by searching databases for MHC ligands and peptide motifs (Rammensee, H., et al. (1999) Immunogenetics, 50:213-219). This information can be used to identify MHC epitopes. Typically, epitopes recognized by MHC class I molecules

comprise between 8 and 11 amino acids while epitopes recognized by MHC class II molecules comprise between 10 to 20 amino acids (Fundamental Immunology, 4th edition, W. E. Paul, ed., Lippincott-Raven Publishers, 1999, Chapter 39, pp 1295-1334). Thus, in some embodiments, MFPs range between 8 to 11. In other embodiments, MFPs range between 10 to 20 amino acids. In other embodiments, MFPs range from 8 to 350 amino acids. In still other embodiments, MFPs range between 20 to 100 amino acids, or from 20 to 135 amino acids, or from 20 to 185 amino acids, or from 20 to 200 amino acids, or from 20 to 250 amino acids, or from 20 to 300 amino acids or from 20 to 350 amino acids. In yet other embodiments, MFPs range from 50 to 100 amino acids, or from 50 to 135 amino acids, or from 50 to 150 amino acids, or from 50 to 175 amino acids, or from 50 to 200 amino acids, or from 50 to 250 amino acids, or from 50 to 350 amino acids, or from 100 to 200 amino acids, or from 100 to 300 amino acids, or from 100 to 350 amino acids.

[080] The MFPs may be unprocessed or processed. As used herein "unprocessed" refers to an MFP that is still associated with the laminin-5 α3 chain. By "processed" herein is meant that the MFP is dissociated from the laminin-5 α3 chain.

[081] FIG. 1B illustrates an exemplary embodiment of MFPs that can be generated from the G4 domain. FIG. 1B depicts 3 MFPs: **MFP 1**, **MFP 2**, And **MFP 3**. Known cleavage sites for bone morphogenetic protein-1 (BMP-1) are indicated by the solid boxes labeled **1a**, **1b**, and **1c** (see U.S. Pat. Pub. No. 2002/0076736). As illustrated in FIG. 1B, the MFPs described herein do not comprise cleavage sites for bone morphogenetic protein-1 (BMP-1) or related BMP-1 proteins. As will be appreciated by a skilled artisan, other MFPs (**MFPs 4**) can be generated from the G4 domain, comprising from 8 up to130 amino acids.

[082] FIG. 1C illustrates an exemplary embodiment of MFPs that can be generated from the G5 domain. As illustrated in FIG. 1C, one MFP can be made, i.e. **MFP 5** spanning the entire G5 domain. In other embodiments one MFP can be made, i.e. **MFP 6**, that spans a subdomain of the G5 domain. Alternatively, a number of MFPs, i.e. **MFPs 7**, can be made comprising from 8 up to 182 amino acids.

[083] FIG. 1D illustrates an exemplary embodiment of MFPs that can be generated from the G5 and the G5 domain. As illustrated in FIG. 1D, one MFP can be made, i.e. **MFP 8**, spanning the G4-G5 domain. As illustrated in FIG. 1D, **MFP 8** does not contain cleavage sites for BMP-1 or related BMP-1 proteins. Alternatively, a number of MFPs, i.e., **MFPs 9**, can be made comprising from 8 up to 315 amino acids.

2.3 Expression Systems

[084] MFSs polynucleotides encoding MFPs can be used to make a variety of expression vectors to express MFPs which can then be used in the diagnostic, screening and therapeutic applications described below. The expression vectors may be either self-replicating extrachromosomal vectors or vectors which integrate into a host genome. Generally, these expression vectors include transcriptional and translational regulatory nucleic acid operably linked to the oligonucleotide encoding the MFP protein. The term "control sequences" refers to DNA sequences necessary for the expression of an operably linked coding sequence in a particular host organism. The control sequences that are suitable for prokaryotes, for example, include a promoter, optionally an operator sequence, and a ribosome binding site. Eukaryotic cells are known to utilize promoters, polyadenylation signals, and enhancers.

[085] An oligonucleotide is "operably linked" when it is placed into a functional relationship with another olignonucleotide sequence. For example, DNA for a presequence or secretory leader is operably linked to DNA for a polypeptide if it is expressed as a preprotein that participates in the secretion of the polypeptide; a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the sequence; or a ribosome binding site is operably linked to a coding sequence if it is positioned so as to facilitate translation. Generally, "operably linked" means that the DNA sequences being linked are contiguous, and, in the case of a secretory leader, contiguous and in reading phase. However, enhancers do not have to be contiguous. Linking is accomplished by ligation at convenient restriction sites. If such sites do not exist, synthetic oligonucleotide adaptors or linkers are used in accordance with conventional practice. The transcriptional

and translational regulatory nucleic acid will generally be appropriate to the host cell used to express the MFP protein; for example, transcriptional and translational regulatory nucleic acid sequences from *Bacillus* are preferably used to express the MFP protein in *Bacillus*. Numerous types of appropriate expression vectors, and suitable regulatory sequences are known in the art for a variety of host cells.

[086] In general, the transcriptional and translational regulatory sequences may include, but are not limited to, promoter sequences, ribosomal binding sites, transcriptional start and stop sequences, translational start and stop sequences, and enhancer or activator sequences. In a preferred embodiment, the regulatory sequences include a promoter and transcriptional start and stop sequences.

[087] Promoter sequences encode either constitutive or inducible promoters. The promoters may be either naturally occurring promoters or hybrid promoters. Hybrid promoters, which combine elements of more than one promoter, are also known in the art, and are useful in the present invention.

[088] In addition, the expression vector may comprise additional elements. For example, the expression vector may have two replication systems, thus allowing it to be maintained in two organisms, for example in mammalian or insect cells for expression and in a procaryotic host for cloning and amplification. Furthermore, for integrating expression vectors, the expression vector contains at least one sequence homologous to the host cell genome, and preferably two homologous sequences which flank the expression construct. The integrating vector may be directed to a specific locus in the host cell by selecting the appropriate homologous sequence for inclusion in the vector. Constructs for integrating vectors are well known in the art.

[089] In addition, in a preferred embodiment, the expression vector contains a selectable marker gene to allow the selection of transformed host cells. Selection genes are well known in the art and will vary with the host cell used.

[090] MFPs are produced by culturing a host cell transformed with an expression vector containing an oligonucleotide encoding an MFP, under the appropriate conditions to induce or cause expression of the MFP. The conditions appropriate for MFP expression will vary with the choice of the expression vector and the host cell, and will be easily ascertained by one skilled in the art through routine experimentation. For example, the use of constitutive promoters in the expression vector will require optimizing the growth and proliferation of the host cell, while the use of an inducible promoter requires the appropriate growth conditions for induction. In addition, in some embodiments, the timing of the harvest is important. For example, the baculoviral systems used in insect cell expression are lytic viruses, and thus harvest time selection can be crucial for product yield.

[091] Appropriate host cells include yeast, bacteria, archaebacteria, fungi, and insect, plant and animal cells, including mammalian cells. Of particular interest are primary human keratinocytes, although other cells also can be used, *i.e. Drosophila melanogaster* cells, *Saccharomyces cerevisiae* and other yeasts, *E. coli*, *Bacillus subtilis*, 293 cells, CHO, other human cell and cell lines.

[092] In some embodiments, the MFPs are expressed in mammalian cells. Mammalian expression systems are also known in the art, and include retroviral systems. A preferred expression vector system is a retroviral vector system such as is generally described in Dajee et al., 2003, *Nature*, 421: 639-643, which is incorporated herein by reference in its entirety. Of particular use as mammalian promoters are the promoters from mammalian viral genes, since the viral genes are often highly expressed and have a broad host range. Examples include, but are not limited to, the SV40 early promoter, mouse mammary tumor virus LTR promoter, adenovirus major late promoter, herpes simplex virus promoter, and the CMV promoter. Typically, transcription termination and polyadenylation sequences recognized by mammalian cells are regulatory regions located 3' to the translation stop codon and thus, together with the promoter elements, flank the coding sequence. Examples of transcription terminator and polyadenlytion signals include those derived form SV40.

[093] For example, the full length laminin –5 α3 cDNA can be ligated into a pENTR1A™ vector (Invitrogen). The full length laminin –5 α3 cDNA can be cleaved and the PCR used to obtain a MF oligonucleotide sequence from the G4 and/or G5 domain. The resulting PCR product can be ligated into a pENTR1A™ vector and the cloning product confirmed by sequencing. The cloned product can then be transferred from the pENTR1A™ vector to a Gateway adapted LSRZ retroviral vector through lambda phage recombination. See Dajee et al., 2003, *Nature*, 421: 639-643.

[094] In some embodiments, MFPs are expressed in bacterial systems. Bacterial expression systems are well known in the art. Promoters from bacteriophage may also be used and are known in the art. In addition, synthetic promoters and hybrid promoters are also useful; for example, the tac promoter is a hybrid of the trp and lac promoter sequences. Furthermore, a bacterial promoter can include naturally occurring promoters of non-bacterial origin that have the ability to bind bacterial RNA polymerase and initiate transcription. In addition to a functioning promoter sequence, an efficient ribosome binding site is desirable. The expression vector may also include a signal peptide sequence that provides for secretion of the MFP in bacteria. The protein is either secreted into the growth media (gram-positive bacteria) or into the periplasmic space, located between the inner and outer membrane of the cell (gram-negative bacteria). The bacterial expression vector may also include a selectable marker gene to allow for the selection of bacterial strains that have been transformed. Suitable selection genes include genes which render the bacteria resistant to drugs such as ampicillin, chloramphenicol, erythromycin, kanamycin, neomycin and tetracycline. Selectable markers also include biosynthetic genes, such as those in the histidine, tryptophan and leucine biosynthetic pathways. These components are assembled into expression vectors. Expression vectors for bacteria are well known in the art, and include vectors for *Bacillus subtilis*, *E. coli*, *Streptococcus cremoris*, and *Streptococcus lividans*, among others. The bacterial expression vectors are transformed into bacterial host cells using techniques well known in the art, such as calcium chloride treatment, electroporation, and others.

[095] The methods of introducing exogenous nucleic acid into mammalian hosts, as well as other hosts, is well known in the art, and will vary with the host cell used. Techniques include dextran-mediated transfection, calcium phosphate precipitation, polybrene mediated transfection, protoplast fusion, electroporation, viral infection, encapsulation of the polynucleotide(s) in liposomes, and direct microinjection of the DNA into nuclei.

[096] MFS's and MFPs can be identified as described in the examples. For example, in a specific embodiment, various oligonucleotides can be generated from the G4 and G5 domain of the human laminin-5 $\alpha 3$ and subcloned into a retroviral vector. The resulting retroviral vectors can be transduced into cell cultures and the cells analyzed for cell scattering and cell migration (see e.g., Examples and FIGS. 5 and 6; see also Ryan, et al., 1994, *J. Biol. Chem.*, 269: 22779-22787). Alternatively, laminin-5 negative primary human keratinocytes co-expressing Ras, a stable NF-κB repressor mutant of IκBα (i.e. IKB), and one or more MFS(s) can be retrovirally transduced and used to regenerate human skin on immune deficient mice (i.e. nude mice). The subsequent development of neoplasms can be monitored and compared to wild type mice (see e.g., Examples, FIG. 7; and Dajee et al., 2003, *Nature*, 421:639-643).

[097] In some embodiments, matrigel, which contains heparin sulfate proteoglycan, is used as a matrix for the suspension of RAS/IKB transformed keratinocytes prior to subcutaneous injection into nude mice. In other embodiments, MFPs can be suspended in matrigel prior to injection of RAS/IKB transformed keratinocytes.

[098] In some embodiments, MFPs are purified or isolated after expression. MFPs may be isolated or purified in a variety of ways known to those skilled in the art depending on what other components are present in the sample. Standard purification methods include electrophoretic, molecular, immunological and chromatographic techniques, including ion exchange, hydrophobic, affinity, and reverse-phase HPLC chromatography, and chromatofocusing. For example, the MFP may be purified using a standard anti-MFP antibody column. Ultrafiltration and diafiltration techniques, in conjunction with protein

concentration, are also useful. For general guidance in suitable purification techniques, see Scopes, R., Protein Purification, Springer-Verlag, NY (1982). The degree of purification necessary will vary depending on the use of the MFP protein. In some instances no purification will be necessary.

2.4 Antisense Sequences

[099] The MFSs and MFPs can be used in a variety of different ways. In some embodiments, MFSs can be used to make antisense therapeutic agents that affect the expression and activity of MFPs. Antisense technology relies on the modulation of expression of a target protein through the specific binding of an antisense sequence to a target sequence encoding the target protein or directing its expression. (See, e.g., Agrawal, S., ed., 1996, *Antisense Therapeutics*, Humana Press Inc., Totawa N.J.; Alama et al. (1997) *Pharmacol Res.* 36(3):171-178; Crooke, S. T., 1997, *Adv. Pharmacol.* 40:1-49; and Lavrosky et al., 1997, *Biochem. Mol. Med.* 62(1):11-22.). Antisense sequences are nucleic acid sequences capable of specifically hybridizing to at least a portion of a target sequence. Antisense sequences can bind to cellular mRNA or genomic DNA, blocking translation or transcription and thus interfering with expression of a targeted protein product. Antisense sequences can be any nucleic acid material, including DNA, RNA, or any nucleic acid mimics or analogs. (See, e.g., Rossi et al., 1991, *Antisense Res. Dev.* 1(3):285-288; Pardridge et al., 1995, *Proc. Nat. Acad. Sci.* 92 (12):5592-5596; Nielsen, P. E. and G. Haaima, 1997, *Chem. Soc. Rev.* 96:73-78; and Lee et al., 1998, *Biochemistry* 37 (3):900-1010.). Delivery of antisense sequences can be accomplished in a variety of ways, such as through intracellular delivery using an expression vector. Site-specific delivery of exogenous genes is also contemplated, such as techniques in which cells are first transfected in culture and stable transfectants are subsequently delivered to the target site.

[0100] Typically, antisense oligonucleotides between 15 to 25 nucleobases or nucleobase analogs are capable of producing the desired therapeutic effect, i.e., direct disruption of translation of an MFP. In addition, chemically reactive groups, such as iron-linked ethylenediamine-tetraacetic acid (EDTA-Fe), can be attached to antisense

oligonucleotides, causing cleavage of the RNA at the site of hybridization. These and other uses of antisense methods to inhibit the *in vitro* translation of genes are well known in the art (see, e.g., Marcus-Sakura (1988) *Anal. Biochem.* 172:289).

[0101] In some embodiments, antisense oligonucleotides are designed such that they disrupt the translation of the laminin-5 α3 chain. In other embodiments, antisense oligonucleotides are designed such that they disrupt the translation of an MFP from the G4 domain or subdomain thereof. In still other embodiments, antisense oligonucleotides are designed such that they disrupt the translation of an MFP from the G5 domain or subdomain thereof. In yet other embodiment, antisense oligonucleotides are designed such that they disrupt the translation of an MFP from the G4 and G5 domain or subdomain thereof.

[0102] Delivery of antisense agents can be achieved intracellularly through using a recombinant expression vector such as a chimeric virus or a colloidal dispersion system which, upon transcription, produces a sequence complementary to at least a portion of the cellular sequence encoding the target protein (see, e.g., Slater et al., 1998, *J. Allergy Cli. Immunol.* 102 (3): 469-475). Delivery of antisense sequences can also be achieved through various viral vectors, including retrovirus and adeno-associated virus vectors. (See, e.g., Miller, 1990, *Blood,* 76: 271; and Uckert and Walther, 1994, *Pharacol. Ther.*, 63(3): 323-347). Suitable viral vectors include, but are not limited to, adenoviruses, herpes viruses, vaccinia, and RNA viruses such as retroviruses.

[0103] Retroviral vectors can be derivatives of murine or avian retrovirus. Retroviral vectors can be made target-specific by inserting, for example, a polynucleotide encoding a protein or proteins such that the desired ligand is expressed on the surface of the viral vector. The ligand can be a glycolipid carbohydrate or protein. Preferred targeting can also be accomplished by using an antibody to target the retroviral vector. Those of skill in the art will know of, or can readily ascertain without undue experimentation, specific polynucleotide sequences which can be inserted into the retroviral genome to allow target

specific delivery of the retroviral vector containing the antisense polynucleotide. See, e.g, WO 91/04753.

[0104] Other delivery mechanisms that can be used for delivery of antisense sequences to target cells include colloidal dispersion and liposome-derived systems, artificial viral envelopes, and other systems available to one of skill in the art (see, e.g., Rossi, 1995, *Br. Med. Bull.* 51 (1): 217-225; Morris et al., 1997, *Nucl. Acids Res.* 25 (14): 2730-2736; Boado et al., 1998, *J. Pharm. Sci.* 87 (11): 1308-1315; and WO 90/10448). For example, delivery systems can make use of macromolecule complexes, nanocapsules, microspheres, beads, and lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes.

2.5 Antibodies

[0105] In some embodiments, the MFPs are used to generate antibodies that can be used in the screening and therapeutic applications described herein. Preferably, the MFP should comprise at least one epitope or determinant. In some embodiments, the epitope is unique; that is, antibodies generated to a unique epitope show little or no cross-reactivity. The term "antibody" can include polyclonal antibodies, monoclonal antibodies, chimeric antibodies, antibody fragments, such as Fab, Fab_2, single chain antibodies (Fv for example) etc., either produced by the modification of whole antibodies or those synthesized *de novo* using recombinant DNA technologies.

[0106] MFPs can be evaluated to determine regions of immunogenicity. As discussed above, methods of analysis and epitope selection are well-known in the art. Analysis and selection can also be accomplished, for example, by various software packages, such as LASERGENE NAVIGATOR software (DNASTAR; Madison, Wis.). The polypeptides or fragments used to induce antibodies should be antigenic, but need not necessarily be biologically active. An antigenic fragment or polypeptide is at least 5 amino acids in length, more preferably, at least 10 amino acids in length, and most preferably, at least 15 amino acids in length. It is preferable that the antibody-inducing fragment or polypeptide is

identical to at least a portion of the amino acid sequence of the G4 and/or G5 domain, or subdomains thereof. It may be useful to conjugate the immunizing agent to a protein known to be immunogenic in the mammal being immunized. Examples of such immunogenic proteins include but are not limited to keyhole limpet hemocyanin, serum albumin, bovine thyroglobulin, and soybean trypsin inhibitor, and antibodies can be produced against the chimeric molecule.

[0107] Methods for the production of antibodies are well-known in the art. For example, various hosts, including goats, rabbits, rats, mice, humans, and others, may be immunized by injection with the MFP or any immunogenic fragment or peptide thereof. Depending on the host species, various adjuvants may be used to increase immunological response. Such adjuvants include, but are not limited to, Freund's adjuvant, mineral gels such as aluminum hydroxide, and surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, KLH, and dinitrophenol. Among adjuvants used in humans, BCG (bacilli Calmette-Guerin) and Corynebacterium parvum are especially preferable.

[0108] Monoclonal and polycolonal antibodies can be prepared using any technique which provides for the production of antibody molecules by continuous cell lines in culture. Techniques for *in vivo* and *in vitro* production are well-known in the art (see, e.g., Pound, J. D., 1998, *Immunochemical Protocols*, Humana Press, Totowa N.J.; Harlow, E. and D. Lane, 1988, Antibodies, A Laboratory Manual, Cold Spring Harbor Laboratory, New York). The production of chimeric antibodies is also well-known, as is the production of single-chain antibodies (see, e.g., Morrison et al., 1984, *Proc. Natl. Acad. Sci.* 81: 6851-6855; Neuberger et al., 1984, *Nature*, 312: 604-608; Takeda et al., 1985, *Nature*, 314: 452-454). Antibodies with related specificity, but of distinct idiotypic composition, may be generated, for example, by chain shuffling from random combinatorial immunoglobin libraries (see, e.g., Burton, 1991, *Proc. Natl. Acad. Sci.* 88: 11120-11123).

[0109] Antibodies may also be produced by inducing *in vivo* production in the lymphocyte population or by screening immunoglobulin libraries or panels of highly

specific binding reagents (see, e.g., Orlandi et al., 1989, *Proc. Natl. Acad. Sci.* 86: 3833-3837; Winter, G. and C. Milstein, 1991, *Nature*, 349: 293-299). Antibody fragments which contain specific binding sites for the target polypeptide may also be generated. Such antibody fragments include, but are not limited to, $F(ab')_2$ fragments, which can be produced by pepsin digestion of the antibody molecule, and Fab fragments, which can be generated by reducing the disulfide bridges of the $F(ab')_2$ fragments. Alternatively, Fab expression libraries may be constructed to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity (see, e.g., Huse et al., 1989, *Science*, 254: 1275-1281).

[0110] In some embodiments, the antibodies are bispecific antibodies. Bispecific antibodies are monoclonal, preferably human or humanized, antibodies that have binding specificities for at least two different antigens. In the present case, one of the binding specificities is for a MFP, and the other one is for any other antigen, such as a cell-surface protein or receptor or receptor subunit, preferably one that is tumor specific.

[0111] In some embodiments, the antibodies to MFPs are humanized antibodies. Humanized forms of non-human (e.g., murine) antibodies are chimeric molecules of immunoglobulins, immunoglobulin chains or fragments thereof (such as Fv, Fab, Fab', $F(ab')_2$ or other antigen binding subsequences of antibodies) which contain minimal sequence derived from non-human immunoglobulin. Humanized antibodies include human immunoglobulins (recipient antibody) in which residues form a complementary determining region (CDR) of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat or rabbit having the desired specificity, affinity and capacity. In some instances, Fv framework residues of the human immunoglobulin are replaced by corresponding non-human residues. Humanized antibodies may also comprise residues which are found neither in the recipient antibody nor in the imported CDR or framework sequences. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human

immunoglobulin and all or substantially all of the framework residues (FR) regions are those of a human immunoglobulin consensus sequence. The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin (Jones et al., 1986, *Nature*, 321: 522-525; Riechmann et al., 1988, *Nature*, 332: 323-329; and Presta, 1992, *Curr. Op. Struct. Biol.*, 2: 593-596).

[0112] Methods for humanizing non-human antibodies are well known in the art. Generally, a humanized antibody has one or more amino acid residues introduced into it from a source which is non-human. These non-human amino acid residues are often referred to as import residues, which are typically taken from an import variable domain. Humanization can be essentially performed following the method of Winter and co-workers (Jones et al., 1986, *Nature*, 321: 522-525; Riechmann et al., 1988, *Nature*, 332: 323-329; Verhoeyen et al., 1988, *Science*, 239: 1534-1536), by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody. Accordingly, such humanized antibodies are chimeric antibodies (U.S. Patent No. 4,816,567), wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some CDR residues and possibly some FR residues are substituted by residues from analogous sites in rodent antibodies.

[0113] Human antibodies can also be produced using various techniques known in the art, including phage display libraries [Hoogenboom and Winter, 1991, *J. Mol. Biol.*, 227: 381; Marks et al., 1991, *J. Mol. Biol.*, 222: 581). The techniques of Cole et al. and Boerner et al. are also available for the preparation of human monoclonal antibodies (Cole et al., 1985, Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, p. 77, and Boerner et al., 1991, *J. Immunol.*, 147(1): 86-95). Similarly, human antibodies can be made by introducing human immunoglobulin loci into transgenic animals, e.g., mice in which the endogenous immunoglobulin genes have been partially or completely inactivated. Upon challenge, human antibody production is observed, which closely resembles that seen in humans in all respects, including gene rearrangement, assembly, and antibody repertoire.

This approach is described, for example, in U.S. Patent Nos. 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; 5,661,016, and in the following scientific publications: Marks et al., 1992, *Bio/Technology*, 10: 779-783; Lonberg et al., 1994, *Nature*, 368: 856-859; Morrison, 1994, *Nature*, 368: 812-13; Fishwild et al., 1996, *Nature Biotechnology*, 14: 845-51; Neuberger, 1996, *Nature Biotechnology*, 14: 826; Lonberg and Huszar, 1995, *Intern. Rev. Immunol.* 13 65-93.

[0114] Antibodies can be tested for anti-MFP activity using a variety of methods well-known in the art. Various techniques may be used for screening to identify antibodies having the desired specificity, including various immunoassays, such as enzyme-linked immunosorbent assays (ELISAs), including direct and ligand-capture ELISAs, radioimmunoassays (RIAs), immunoblotting, and fluorescent activated cell sorting (FACS). Numerous protocols for competitive binding or immunoradiometric assays, using either polyclonal or monoclonal antibodies with established specificities, are well known in the art (see, e.g., Harlow and Lane, *supra*). Such immunoassays typically involve the measurement of complex formation between the target polypeptide and a specific antibody. A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering epitopes on the target polypeptide is preferred, but other assays, such as a competitive binding assay, may also be employed (see, e.g. Maddox et al., 1983, *J Exp Med*, 158: 1211).

[0115] Once made, the antibodies can be used to identify MFPs in a sample, e.g., from biopsied tissue, etc. The amount of MFPs or mRNAs encoding MRPs can be determined using methods well known in the art, including but not limited to, quantitative image analysis, and reverse transcriptase polymerase chain reaction (RT-PCR) using portions of the biopsied tissue. Quantitation of mRNA corresponding to MFPs, can be determined by a competition reaction using equal volumes of the patient sample run against a series of decreasing known concentrations, e.g., of a mimic or mutant cDNA fragment.

[0116] MFP antibodies as described herein, are capable of specifically binding to MFPs. By "specifically binding" herein is meant that the antibodies bind to the protein

with a binding constant in the range of at least 10^{-4}- 10^{-9} M^{-1}, preferably in the range of 10^{-4}- 10^{-6} M^{-1}, with a preferred range being 10^{-7} - 10^{-9} M^{-1}.

[0117] In some embodiments, antibodies to MFPs are capable of reducing or eliminating the biological activity or function of the MFP(s). That is, the addition of anti-MFP antibodies (i.e., polyclonal or monoclonal) to SCC or neoplastic epithelial cells expressing a MFP reduces or eliminates the MFP activity. Generally, at least a 25% decrease in activity is preferred, with at least about 50% being particularly preferred and about a 95-100% decrease being especially preferred.

[0118] In some embodiments, antibodies to MFPs are conjugated to a therapeutic moiety. For example, the therapeutic moiety can be an agent inhibit enzymatic activity such as protease or protein kinase activity associated with SCC. In other embodiments, the therapeutic moiety can be a cytotoxic agent. Cytotoxic agents are numerous and varied and include, but are not limited to, radiochemicals, cytotoxic drugs or toxins or active fragments of such toxins. Suitable toxins and their corresponding fragments include diphtheria A chain, exotoxin A chain, ricin A chain, abrin A chain, curcin, crotin, phenomycin, enomycin and the like.

2.6 Diagnosis and Therapy

[0119] The MFSs and MFPs can be used in a variety of different ways. For example, the MFSs and MFPs can be used in diagnostic assays, screening assays, and in therapeutic application. In some embodiments, the MFPS and antibodies to MFPs are used diagnostic markers for the detection of SCC. Detection of MFPs in putative SCC tissue or patients allows for a determination or diagnosis of SCC. To detect or diagnose SCC, baseline values for the expression or activity of MFPs are established in order to provide a basis for the diagnosis and/or prognosis of SCC in a patient. In some embodiments, this is accomplished by combining body fluids, tissue biopsies, or cell extracts taken from normal subjects with one or more antibody(ies) to a MFP under conditions suitable for complex formation. Such conditions are well known in the art. The amount of standard complex

formation may be quantified by comparing levels of antibody-target complex in the normal sample with a dilution series of positive controls, in which a known amount of antibody is combined with known concentrations of purified MFP. Standard values obtained from normal samples may be compared with values obtained from samples from subjects suspected of having SCC. Deviation between standard and subject values establishes the presence of or predisposition to the disease state.

[0120] In other embodiments, the expression levels of genes are determined for different cellular states in the SCC phenotype; that is, the expression levels of genes in normal tissue and in SCC tissue are evaluated to provide expression profiles. An expression profile of a particular cell state or point of development is essentially a "fingerprint" of the state; while two states may have any particular gene similarly expressed, the evaluation of a number of genes simultaneously allows the generation of a gene expression profile that is unique to the state of the cell. By comparing expression profiles of cells in different states, information regarding which genes are important (including both up- and down-regulation of genes) in each of these states is obtained. Then, diagnosis may be done or confirmed: does tissue from a particular patient have the gene expression profile of normal or SCC tissue.

[0121] "Differential expression," or grammatical equivalents as used herein, refers to both qualitative as well as quantitative differences in the genes' temporal and/or cellular expression patterns within and among the cells. Thus, a differentially expressed gene can qualitatively have its expression altered, including an activation or inactivation, in, for example, normal versus lymphoma tissue. That is, genes may be turned on or turned off in a particular state, relative to another state. As is apparent to the skilled artisan, any comparison of two or more states can be made. Such a qualitatively regulated gene will exhibit an expression pattern within a state or cell type which is detectable by standard techniques in one such state or cell type, but is not detectable in both. Alternatively, the determination is quantitative in that expression is increased or decreased; that is, the expression of the gene is either upregulated, resulting in an increased amount of transcript,

or downregulated, resulting in a decreased amount of transcript. The degree to which expression differs need only be large enough to quantify via standard characterization techniques as outlined below, such as by use of Affymetrix GeneChip™ expression arrays, Lockhart, Nature Biotechnology, 14:1675-1680 (1996), hereby expressly incorporated by reference. Other techniques include, but are not limited to, quantitative reverse transcriptase PCR, Northern analysis and RNase protection. As outlined above, preferably the change in expression (i.e. upregulation or downregulation) is at least about 50%, more preferably at least about 100%, more preferably at least about 150%, more preferably, at least about 200%, with from 300 to at least 1000% being especially preferred.

[0122] As will be appreciated by those in the art, this may be done by evaluation at either the gene transcript, or the protein level; that is, the amount of gene expression may be monitored using nucleic acid probes to the DNA or RNA equivalent of the gene transcript, and the quantification of gene expression levels, or, alternatively, the final gene product itself (protein) can be monitored, for example through the use of antibodies to the MF protein and standard immunoassays (ELISAs, etc.) or other techniques, including mass spectroscopy assays, 2D gel electrophoresis assays, etc. Thus, the proteins corresponding to MF genes, i.e. those identified as being important in a SCC phenotype, can be evaluated in a SCC diagnostic test.

[0123] Numerous methods known to those of ordinary skill in the art find use in diagnosisng SCC. For example, in some embodiments, proteins can be obtained from a sample or a patient are separated by electrophoresis on a gel (typically a denaturing and reducing protein gel, but may be any other type of gel including isoelectric focusing gels and the like). Following separation of the proteins, MFPs can be detected by immunoblotting with antibodies raised against the MFPs. Methods of immunoblotting are well known to those of ordinary skill in the art.

[0124] In some embodiments, antibodies to the MFPs find use in *in situ* imaging techniques. In this method cells are contacted with from one to many antibodies to MFP(s). Following washing to remove non-specific antibody binding, the presence of the antibody

or antibodies is detected. In one embodiment the antibody is detected by incubating with a secondary antibody that contains a detectable label. In another method the primary antibody to the MFP(s) contains a detectable label. In another preferred embodiment each one of multiple primary antibodies contains a distinct and detectable label. This method finds particular use in simultaneous screening for a plurality of MFPs. As will be appreciated by one of ordinary skill in the art, numerous other histological imaging techniques are useful in the invention.

[0125] In some embodiments the label is detected in a fluorometer which has the ability to detect and distinguish emissions of different wavelengths. In addition, a fluorescence activated cell sorter (FACS) can be used in the method.

[0126] In some embodiments, *in situ* hybridization of labeled MF nucleic acid probes to tissue arrays is done. For example, arrays of tissue samples, including SCC tissue and/or normal tissue, are made. *In situ* hybridization as is known in the art can then be done.

[0127] It is understood that when comparing the expression fingerprints between an individual and a standard, the skilled artisan can make a diagnosis as well as a prognosis. It is further understood that the genes which indicate the diagnosis may differ from those which indicate the prognosis.

[0128] In a preferred embodiment, the MF proteins, antibodies, nucleic acids, and cells containing MF sequences are used in prognosis assays. In some embodiments, gene expression profiles can be generated that correlate to SCC severity, in terms of long term prognosis. Again, this may be done on either a protein or gene level, with the use of genes being preferred. In some embodiments, MF probes are attached to solid supports for the detection and quantification of MF sequences in a tissue or patient. The assays proceed as outlined for diagnosis.

[0129] The efficacy of therapeutic agents, such as antibodies and/or other candidate drugs also can be determined using the diagnostic assays described above. As will be appreciated by a person of skill in the art, assays to determine the efficacy of a therapeutic

agent require the establishment of baseline values. In some embodiments, this is accomplished by combining body fluids, tissue biopsies, or cell extracts taken from a patient with SCC prior to treatment with the candidate drug with one or more antibody(ies) to a MFP under conditions suitable for complex formation. Such conditions are well known in the art. The amount of standard complex formation may be quantified by comparing levels of antibody-target complex in the normal sample with a dilution series of positive controls, in which a known amount of antibody is combined with known concentrations of purified MFP. Standard values obtained from a patient before treatment may be compared with values obtained from a patient after treatment. Deviation between standard and subject values establishes the efficacy of the drug.

2.7 Screening Assays

[0130] In some embodiments, the MF proteins, antibodies, nucleic acids, and cells containing the MF proteins are used in screening assays. For example, screens for agents that modulate the SCC phenotype can be run. This can be done by screening for modulators of gene expression or for modulators of protein activity at the individual gene or protein level or by evaluating the effect of drug candidates on a "gene expression profile". In some embodiments, the expression profiles are used in conjunction with high throughput screening techniques to allow monitoring for expression profile genes after treatment with a candidate agent (see Zlokarnik, et al., 1998, *Science*, 279: 84-8).

[0131] "Modulation" includes both an increase and a decrease in gene expression or activity. The preferred amount of modulation will depend on the original change of the gene expression in normal versus tumor tissue, with changes of at least 10%, preferably 50%, more preferably 100-300%, and in some embodiments 300-1000% or greater. If a gene exhibits a 4 fold increase in tumor compared to normal tissue, a decrease of about four fold is desired; a 10 fold decrease in tumor compared to normal tissue gives a 10 fold increase in expression for a candidate agent is desired, etc.

[0132] As will be appreciated by those in the art, this may be done by evaluation at either the gene or the protein level; that is, the amount of gene expression may be monitored using nucleic acid probes and the quantification of gene expression levels, or, alternatively, the level of the gene product itself can be monitored, for example through the use of antibodies to the MFPd and standard immunoassays. Alternatively, binding and bioactivity assays with the protein may be done as outlined below.

[0133] In some embodiments, gene expression monitoring is done and a number of genes, i.e. an expression profile, are monitored simultaneously. If desired, multiple protein expression monitoring can be done as well. In embodiments monitoring multiple genes or proteins, the corresponding MF probes are immobilized to solid supports. It is understood that immobilization can occur by any means, including for example; by covalent attachment, by electrostatic immobilization, by attachment through a ligand/ligand interaction, by contact or by depositing on the surface. "Solid support" or "solid substrate" refers to any solid phase material upon which a MF sequence, MFP, or antibody is synthesized, attached, ligated or otherwise immobilized. A solid support may be composed of organic polymers such as polystyrene, polyethylene, polypropylene, polyfluoroethylene, polyethyleneoxy, and polyacrylamide, as well as co-polymers and grafts thereof. A solid support may also be inorganic, such as glass, silica, controlled-pore-glass (CPG), or reverse-phase silica. The configuration of a solid support may be in the form of beads, spheres, particles, granules, a gel, or a surface. Surfaces may be planar, substantially planar, or non-planar. Solid supports may be porous or non-porous, and may have swelling or non-swelling characteristics. A solid support may be configured in the form of a well, depression or other container, vessel, feature or location. A plurality of solid supports may be configured in an array at various locations, addressable for robotic delivery of reagents, or by detection means including scanning by laser illumination and confocal or deflective light gathering.

[0134] Generally, a candidate bioactive agent is added prior to analysis. The term "candidate bioactive agent" or "drug candidate" or grammatical equivalents as used herein

describes any molecule, e.g., protein, oligopeptide, small organic or inorganic molecule, polysaccharide, polynucleotide, etc., to be tested for bioactive agents that are capable of directly or indirectly altering either the SCC phenotype, binding to and/or modulating the bioactivity of an MFP, or the expression of a MF sequence. In a particularly preferred embodiment, the candidate agent suppresses a SCC phenotype, for example to a normal tissue fingerprint. Generally a plurality of assay mixtures are run in parallel with different agent concentrations to obtain a differential response to the various concentrations. Typically, one of these concentrations serves as a negative control, i.e., at zero concentration or below the level of detection.

[0135] In one aspect, a candidate agent will neutralize the effect of an MFP. By "neutralize" is meant that activity of a protein is either inhibited or counter acted against so as to have substantially no effect on a cell.

[0136] Candidate agents encompass numerous chemical classes, though typically they are organic or inorganic molecules, preferably small organic compounds having a molecular weight of more than 100 and less than about 2,500 daltons. Preferred small molecules are less than 2000, or less than 1500 or less than 1000 or less than 500 D. Candidate agents comprise functional groups necessary for structural interaction with proteins, particularly hydrogen bonding, and typically include at least an amine, carbonyl, hydroxyl or carboxyl group, preferably at least two of the functional chemical groups. The candidate agents often comprise cyclical carbon or heterocyclic structures and/or aromatic or polyaromatic structures substituted with one or more of the above functional groups. Candidate agents are also found among biomolecules including peptides, proteins, saccharides, fatty acids, steroids, purines, pyrimidines, derivatives, structural analogs or combinations thereof.

[0137] Candidate agents are obtained from a wide variety of sources including libraries of synthetic or natural compounds. For example, numerous means are available for random and directed synthesis of a wide variety of organic compounds and biomolecules, including expression of randomized oligonucleotides. Alternatively, libraries of natural compounds

in the form of bacterial, fungal, plant and animal extracts are available or readily produced. Additionally, natural or synthetically produced libraries and compounds are readily modified through conventional chemical, physical and biochemical means. Known pharmacological agents may be subjected to directed or random chemical modifications, such as acylation, alkylation, esterification, amidification to produce structural analogs.

[0138] In assays for altering the expression profile of one or more MF sequences, after the candidate agent has been added and the cells allowed to incubate for some period of time, the sample containing the MF sequences to be analyzed is added to a solid support. If required, the MF sequence is prepared using known techniques. For example, the sample may be treated to lyse the cells, using known lysis buffers, electroporation, etc., with purification and/or amplification such as PCR occurring as needed, as will be appreciated by those in the art.

[0139] Generally, one of the assay components is labeled to provide a means of detecting the binding complex of interest. By "labeled" herein is meant that a compound has at least one element, isotope or chemical compound attached to enable the detection of the compound. In general, labels fall into three classes: a) isotopic labels, which may be radioactive or heavy isotopes; b) immune labels, which may be antibodies or antigens; and c) colored or fluorescent dyes. The labels may be incorporated into the MF nucleic acids, proteins and antibodies at any position. For example, the label should be capable of producing, either directly or indirectly, a detectable signal. The detectable moiety may be a radioisotope, such as ^{3}H, ^{14}C, ^{32}P, ^{35}S, or ^{125}I, a fluorescent or chemiluminescent compound, such as fluorescein isothiocyanate, rhodamine, or luciferin, or an enzyme, such as alkaline phosphatase, beta-galactosidase or horseradish peroxidase. Any method known in the art for conjugating the antibody to the label may be employed, including those methods described by Hunter et al., 1962, *Nature*, 144: 945; David et al., 1974, *Biochemistry*, 13: 1014; Pain et al., 1981, *J. Immunol. Meth.*, 40: 219; and Nygren, 1982, *J. Histochem. and Cytochem.*, 30: 407. The label also can be an enzyme, such as, alkaline phosphatase or horseradish peroxidase, which when provided with an appropriate substrate produces a

product that can be detected. Alternatively, the label can be a labeled compound or small molecule, such as an enzyme inhibitor, that binds but is not catalyzed or altered by the enzyme. The label also can be a moiety or compound, such as, an epitope tag or biotin which specifically binds to streptavidin. For the example of biotin, the streptavidin is labeled as described above, thereby, providing a detectable signal for the bound target sequence. As known in the art, unbound labeled streptavidin is removed prior to analysis.

[0140] As will be appreciated by those in the art, these assays can be direct hybridization assays or can comprise "sandwich assays", which include the use of multiple probes, as is generally outlined in U.S. Patent Nos. 5,681,702, 5,597,909, 5,545,730, 5,594,117, 5,591,584, 5,571,670, 5,580,731, 5,571,670, 5,591,584, 5,624,802, 5,635,352, 5,594,118, 5,359,100, 5,124,246 and 5,681,697, all of which are hereby incorporated by reference.

[0141] A variety of hybridization conditions may be used in the present invention, including high, moderate and low stringency conditions as outlined above. The assays are generally run under stringency conditions which allows formation of the label probe hybridization complex only in the presence of target. Stringency can be controlled by altering a step parameter that is a thermodynamic variable, including, but not limited to, temperature, formamide concentration, salt concentration, chaotropic salt concentration pH, organic solvent concentration, etc.

[0142] These parameters may also be used to control non-specific binding, as is generally outlined in U.S. Patent No. 5,681,697. Thus it may be desirable to perform certain steps at higher stringency conditions to reduce non-specific binding.

[0143] The reactions outlined herein may be accomplished in a variety of ways, as will be appreciated by those in the art. Components of the reaction may be added simultaneously, or sequentially, in any order, with preferred embodiments outlined below. In addition, the reaction may include a variety of other reagents may be included in the assays. These include reagents like salts, buffers, neutral proteins, e.g. albumin, detergents,

etc which may be used to facilitate optimal hybridization and detection, and/or reduce non-specific or background interactions. Also reagents that otherwise improve the efficiency of the assay, such as protease inhibitors, nuclease inhibitors, anti-microbial agents, etc., may be used, depending on the sample preparation methods and purity of the target. In addition, either solid phase or solution based (i.e., kinetic PCR) assays may be used.

[0144] Once the assay is run, the data is analyzed to determine the expression levels, and changes in expression levels as between states, of individual genes, or individual proteins, forming an expression profile.

[0145] In some embodiments, screening is done to alter the biological function of the expression product of an MF gene. Again, having identified the importance of a gene in a particular state, screening for agents that bind and/or modulate the biological activity of the gene product can be run as is more fully outlined below.

[0146] In some embodiments, screens are designed to first find candidate agents that can bind to MF proteins, and then these agents may be used in assays that evaluate the ability of the candidate agent to modulate the MFP activity and the SCC phenotype. As will be appreciated by those in the art, there are a number of different assays which may be run; binding assays and activity assays.

[0147] In some embodiments, binding assays are done. In general, purified or isolated MFPs are used. The methods comprise combining a MFP and a candidate bioactive agent, and determining the binding of the candidate agent to the MFP. Generally, the MFP or the candidate agent is non-diffusably bound to a solid support having isolated sample receiving areas (e.g. a microtiter plate, an array, etc.). Microtiter plates and arrays are especially convenient because a large number of assays can be carried out simultaneously, using small amounts of reagents and samples. The particular manner of binding of the composition is not crucial so long as it is compatible with the reagents, maintains the activity of the composition and is nondiffusable. Preferred methods of binding include the use of antibodies (which do not sterically block either the ligand binding site or activation

sequence when the protein is bound to the support), direct binding to "sticky" or ionic supports, chemical crosslinking, the synthesis of the protein or agent on the surface, etc. Following binding of the protein or agent, excess unbound material is removed by washing. The sample receiving areas may then be blocked through incubation with bovine serum albumin (BSA), casein or other innocuous protein or other moiety.

[0148] In some embodiments, the MFP is bound to the support, and a candidate bioactive agent is added to the assay. Alternatively, the candidate agent is bound to the support and the MFP is added. Novel binding agents include specific antibodies, non-natural binding agents identified in screens of chemical libraries, peptide analogs, etc. Of particular interest are screening assays for agents that have a low toxicity for human cells. A wide variety of assays may be used for this purpose, including labeled *in vitro* protein-protein binding assays, electrophoretic mobility shift assays, immunoassays for protein binding, functional assays (phosphorylation assays, etc.) and the like.

[0149] The determination of the binding of the candidate bioactive agent to the MFP may be done in a number of ways. In a preferred embodiment, the candidate bioactive agent is labeled, and binding determined directly. For example, this may be done by attaching all or a portion of the MFP to a solid support, adding a labeled candidate agent (for example a fluorescent label), washing off excess reagent, and determining whether the label is present on the solid support. Various blocking and washing steps may be utilized as is known in the art.

[0150] In some embodiments, only one of the components is labeled. For example, the proteins (or proteinaceous candidate agents) may be labeled at tyrosine positions using ^{125}I, or with fluorophores. Alternatively, more than one component may be labeled with different labels; using ^{125}I for the proteins, for example, and a fluorophor for the candidate agents.

[0151] In some embodiments, the binding of the candidate bioactive agent is determined through the use of competitive binding assays. In this embodiment, the

competitor is a binding moiety known to bind to the MFP, such as an antibody, peptide, binding partner, ligand, etc. Under certain circumstances, there may be competitive binding as between the bioactive agent and the binding moiety, with the binding moiety displacing the bioactive agent.

[0152] In some embodiments, the candidate bioactive agent is labeled. Either the candidate bioactive agent, or the competitor, or both, is added first to the protein for a time sufficient to allow binding, if present. Incubations may be performed at any temperature which facilitates optimal activity, typically between 4 and 40°C. Incubation periods are selected for optimum activity, but may also be optimized to facilitate rapid high through put screening. Typically between 0.1 and 1 hour will be sufficient. Excess reagent is generally removed or washed away. The second component is then added, and the presence or absence of the labeled component is followed, to indicate binding.

[0153] In some embodiments, the competitor is added first, followed by the candidate bioactive agent. Displacement of the competitor is an indication that the candidate bioactive agent is binding to the MFP and thus is capable of binding to, and potentially modulating, the activity of the MFP. In this embodiment, either component can be labeled. Thus, for example, if the competitor is labeled, the presence of label in the wash solution indicates displacement by the agent. Alternatively, if the candidate bioactive agent is labeled, the presence of the label on the support indicates displacement.

[0154] In other embodiments, the candidate bioactive agent is added first, with incubation and washing, followed by the competitor. The absence of binding by the competitor may indicate that the bioactive agent is bound to the MFP with a higher affinity. Thus, if the candidate bioactive agent is labeled, the presence of the label on the support, coupled with a lack of competitor binding, may indicate that the candidate agent is capable of binding to the MFP.

[0155] In some embodiments, the methods comprise differential screening to identity bioactive agents that are capable of modulating the activity of the MFPs. In this

embodiment, the methods comprise combining a MFP and a competitor in a first sample. A second sample comprises a candidate bioactive agent, a MFP and a competitor. The binding of the competitor is determined for both samples, and a change, or difference in binding between the two samples indicates the presence of an agent capable of binding to the MFP and potentially modulating its activity. That is, if the binding of the competitor is different in the second sample relative to the first sample, the agent is capable of binding to the MFP.

[0156] In some embodiments, methods for screening for bioactive agents capable of modulating the activity of a MFP in a cell are provided. The methods comprise adding a candidate bioactive agent, as defined above, to a cell comprising MFPs. Typically, laminin-5 negative primary human keratinocytes are used. The cells can also contain recombinant nucleic acids that encode MF sequences, Ras and a stable NF-κB repressor mutant of IκBα (i.e. IKB) (see Dajee et al., 2003, *Nature*, 421: 630-643). Methods for culturing cells and for assaying cell scattering, adhesion and migration are described in Russell et al., 2003, J. Cell Sci., 116: 3543-3556, the entire contents of which are incorporated herein by reference.

[0157] In some embodiments, candidate agents can be introduced into immunodeficient mice that can subsequently be challenged with a MFPs and monitored for the development of tumors. For example, intraperitoneal injections of antibodies against one or more MFPS can be given to mice bearing human foreskin xenografts (see Examples; and Li et al., 2003, EMBO J., 22: 2400-2410). The mice can then be challenged with Ras/IKB transformed human keratinocytes and monitored for tumor growth and histology as described in Dajee et al., 2003, *Nature*, 421: 630-643.

[0158] Positive controls and negative controls may be used in the assays. Preferably all control and test samples are performed in at least triplicate to obtain statistically significant results. Incubation of all samples is for a time sufficient for the binding of the agent to the protein. Following incubation, all samples are washed free of non-specifically bound material and the amount of bound, generally labeled agent determined. For example, where

a radiolabel is employed, the samples may be counted in a scintillation counter to determine the amount of bound compound.

[0159] A variety of other reagents may be included in the screening assays. These include reagents like salts, neutral proteins, e.g. albumin, detergents, etc which may be used to facilitate optimal protein-protein binding and/or reduce non-specific or background interactions. Also reagents that otherwise improve the efficiency of the assay, such as protease inhibitors, nuclease inhibitors, anti-microbial agents, etc., may be used. The mixture of components may be added in any order that provides for the requisite binding.

[0160] In one aspect, the assays are evaluated in the presence or absence or previous or subsequent exposure of physiological signals, for example hormones, antibodies, peptides, antigens, cytokines, growth factors, action potentials, pharmacological agents including chemotherapeutics, radiation, carcinogenics, or other cells (i.e. cell-cell contacts). In another example, the determinations are determined at different stages of the cell cycle process.

2.8 Pharmaceutical Compositions

[0161] Bioactive agents having pharmacological activity are identified as described above. By "pharmacological activity" herein is meant that the compounds are able to enhance or interfere with the activity of MFPs. The compounds having the desired pharmacological activity may be administered in a physiologically acceptable carrier to a patient. A "patient" includes both humans and other animals, particularly mammals, and domestic animals. Thus, the methods are applicable to both human therapy and veterinary applications.

[0162] In some embodiments, bioactive agents include antibodies that recognize MFPs and that have been demonstrated to inhibit or modulate SCC as described herein. In other embodiments, bioactive agents include antisense compositions. These agents can be delivered directly or in pharmaceutical compositions along with suitable carriers or excipients, as well known in the art. Present methods of treatment include embodiments

providing for administration of an effective amount of a compound or agent that inhibits the activity or expression of a MFP to a patient in need of treatment.

[0163] An effective amount of such agents can readily be determined by routine experimentation, as can the most effective and convenient route of administration and the most appropriate formulation. Various formulations and drug delivery systems are available in the art. (See, e.g., Remington's Pharmaceutical Sciences, supra.)

[0164] Suitable routes of administration may, for example, include oral, rectal, transmucosal, transdermal, nasal, or intestinal administration and parenteral delivery, including intramuscular, subcutaneous, intramedullary injections, as well as intrathecal, direct intraventricular, intravenous, intraperitoneal, intranasal, or intraocular injections. The agent or composition thereof may be administered in a local rather than a systemic manner. For example, a suitable agent can be delivered via injection or in a targeted drug delivery system, such as a depot or sustained release formulation.

[0165] The pharmaceutical compositions may be manufactured by any of the methods well-known in the art, such as by conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping, or lyophilizing processes. The compositions can include one or more physiologically acceptable carriers such as excipients and auxiliaries that facilitate processing of active molecules into preparations for pharmaceutical use. Proper formulation is dependent upon the route of administration chosen.

[0166] For example, for injection, the composition may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks's solution, Ringer's solution, or physiological saline buffer. For transmucosal or nasal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art. For oral administration, the agents can be formulated readily by combining the active agents with pharmaceutically acceptable carriers well known in the art. Such carriers enable the agents of the invention to be

formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions and the like, for oral ingestion by a subject. The agents may also be formulated in rectal compositions such as suppositories or retention enemas, e.g., containing conventional suppository bases such as cocoa butter or other glycerides.

[0167] Pharmaceutical preparations for oral use can be obtained as solid excipients, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are, in particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations such as, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carboxymethylcellulose, and/or polyvinylpyrrolidone (PVP). If desired, disintegrating agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate.

[0168] Dragee cores are provided with suitable coatings. For this purpose, concentrated sugar solutions may be used, which may optionally contain gum arabic, talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for identification or to characterize different combinations of active agent doses.

[0169] Pharmaceutical preparations for oral administration include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. The push-fit capsules can contain the active ingredients in admixture with filler such as lactose, binders such as starches, and/or lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active agents may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilizers may be added. All formulations for oral administration should be in dosages suitable for such administration.

[0170] For administration by inhalation, the agents can be conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebuliser, with the use of a suitable propellant, e.g., dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide, or any other suitable gas. In the case of a pressurized aerosol, the appropriate dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges for use in an inhaler or insufflator may be formulated. These typically contain a powder mix of the agent and a suitable powder base such as lactose or starch.

[0171] Compositions formulated for parenteral administration by injection, e.g., by bolus injection or continuous infusion can be presented in unit dosage form, e.g. in ampoules or in multi-dose containers, with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Formulations for parenteral administration include aqueous solutions of the compound or agent to be administered, including in water-soluble form.

[0172] Suspensions of the active agents may also be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil and synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Aqueous injection suspensions may contain substances that increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Optionally, the suspension may also contain suitable stabilizers or agents that increase the solubility of the agents to allow for the preparation of highly concentrated solutions. Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, e.g., sterile pyrogen-free water, before use.

[0173] As mentioned above, the compositions can also be formulated as a depot preparation. Such long acting formulations may be administered by implantation (for example, subcutaneously or intramuscularly) or by intramuscular injection. Thus, for example, the present agents may be formulated with suitable polymeric or hydrophobic

materials (for example as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

[0174] Suitable carriers for the hydrophobic molecules of the invention are well-known in the art and include co-solvent systems comprising, for example, benzyl alcohol, a nonpolar surfactant, a water-miscible organic polymer, and an aqueous phase. The co-solvent system may be the VPD co-solvent system. VPD is a solution of 3% w/v benzyl alcohol, 8% w/v of the nonpolar surfactant polysorbate 80, and 65% w/v polyethylene glycol 300, made up to volume in absolute ethanol. The VPD co-solvent system (VPD:5W) consists of VPD diluted 1:1 with a 5% dextrose in water solution. This co-solvent system is effective in dissolving hydrophobic agents and produces low toxicity upon systemic administration. Naturally, the proportions of a co-solvent system may be varied considerably without destroying its solubility and toxicity characteristics. Furthermore, the identity of the co-solvent components may be varied. For example, other low-toxicity nonpolar surfactants may be used instead of polysorbate 80, the fraction size of polyethylene glycol may be varied, other biocompatible polymers may replace polyethylene glycol, e.g. polyvinyl pyrrolidone, and other sugars or polysaccharides may substitute for dextrose.

[0175] Alternatively, other delivery systems for hydrophobic molecules may be employed. Liposomes and emulsions are well known examples of delivery vehicles or carriers for hydrophobic drugs. Liposomal delivery systems are discussed above in the context of gene-delivery systems. Certain organic solvents such as dimethylsulfoxide also may be employed, although usually at the cost of greater toxicity. Additionally, the agents may be delivered using sustained-release systems, such as semi-permeable matrices of solid hydrophobic polymers containing the effective amount of the composition to be administered. Various sustained-release materials are established and available to those of skill in the art. Sustained-release capsules may, depending on their chemical nature, release the agents for a few weeks up to over 100 days. Depending on the chemical nature and the

biological stability of the therapeutic reagent, additional strategies for protein stabilization may be employed.

[0176] For any composition employed herein, a therapeutically effective dose can be estimated initially using a variety of techniques well-known in the art. For example, in a cell culture assay, a dose can be formulated in animal models to achieve a circulating concentration range that includes the IC_{50} as determined in cell culture. Where inhibition of MFP activity is desired, the concentration of the test agent that achieves a half-maximal inhibition of MFP activity can be determined. Dosage ranges appropriate for human subjects can be determined, using data obtained from cell culture assays and other animal studies.

[0177] A therapeutically effective dose of an agent refers to that amount of the agent that results in amelioration of symptoms or a prolongation of survival in a subject. Toxicity and therapeutic efficacy of such molecules can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., by determining the LD_{50} (the dose lethal to 50% of the population) and the ED_{50} (the dose therapeutically effective in 50% of the population). The dose ratio of toxic to therapeutic effects is the therapeutic index, which can be expressed as the ratio LD_{50}/ ED_{50}. Agents that exhibit high therapeutic indices are preferred.

[0178] Dosages preferably fall within a range of circulating concentrations that includes the ED_{50} with little or no toxicity. Dosages may vary within this range depending upon the dosage form employed and the route of administration utilized. T he exact formulation, route of administration, and dosage should be chosen, according to methods known in the art, in view of the specifics of a subject's condition.

[0179] Dosage amount and interval may be adjusted individually to provide plasma levels or tissue levels of the active moiety which are sufficient to affect the expression or activity of MFPs, as desired, i.e. minimal effective concentration (MEC). The MEC will vary for each agent but can be estimated from, for example, *in vitro* data, such as the

concentration necessary to achieve 50-90% inhibition of MFP activity using the assays described herein. Dosages necessary to achieve the MEC will depend on individual characteristics and route of administration. Agents or compositions thereof should be administered using a regimen which maintains plasma levels above the MEC for about 10-90% of the duration of treatment, preferably about 30-90% of the duration of treatment, and most preferably between 50-90%. In cases of local administration or selective uptake, the effective local concentration of the drug may not be related to plasma concentration.

[0180] The amount of agent or composition administered will, of course, be dependent on a variety of factors, including the sex, age, and weight of the subject being treated, the severity of the affliction, the manner of administration, and the judgment of the prescribing physician.

[0181] The present compositions may, if desired, be presented in a pack or dispenser device containing one or more unit dosage forms containing the active ingredient. Such a pack or device may, for example, comprise metal or plastic foil, such as a blister pack. The pack or dispenser device may be accompanied by instructions for administration. Compositions comprising a agent of the invention formulated in a compatible pharmaceutical carrier may also be prepared, placed in an appropriate container, and labeled for treatment of an indicated condition. Suitable conditions indicated on the label may include treatment of disorders or diseases, such as squamous cell carcinoma or other cancers and conditions associated with altered expression of MFPs.

3. Examples

Example 1. Requirement for G4 and/or G5 Domains in SCC Tumors

[0182] Laminin-5 undergoes processing of both its γ2 and α3 chains. As the α3 chain contains the primary integrin binding site(s), we performed further studies to examine the functional effects of α3 chain processing on SCC tumor development. We created truncations at the following sites: 1) amino acid residue 1337 (1337Tr), and 2) at amino acid residue 1450 (1450Tr). Keratinocytes from a junctional epidermolysis bullosa (JEB)

patient with absent laminin α3 chain expression were transduced with LZRS retroviral vectors containing full length, 1450Tr or 1337Tr cDNA (Matsui et al., 1998, J. Exp. Med., 187: 1273-83). Each of the cDNAs restored trimeric laminin-5 expression in treated JEB keratinocytes, and each cDNA produced comparable levels of secreted laminin-5, as assessed by Western blot using laminin α3 specific antibody. While JEB keratinocytes with no laminin-5 expression (LacZ) were rounded, WT, 1337Tr and 1450Tr expressing keratinocytes showed flattening and spreading. While laminin-5 negative JEB keratinocytes (LacZ) were hypoproliferative, 1337Tr, and 1450Tr showed normal levels of proliferation, comparable to that of wild type (FIG. 5).

[0183] Because laminin-5 processing is closely tied to migration, we studied the 1337Tr mutant in more detail, as truncation at this position simulated the effects of processing *in vivo*. We found that 1337Tr cells were capable of migration, in fact, 1337Tr cells migrated more efficiently in scratch assays compared to cells expressing wild type α3 chain (FIG. 6).

[0184] We have previously described a model of human SCC which is obtained from SQ injection of Ras/IKB over expressing human keratinocytes in nude mice (Dajee et al., 2003, Nature, 421: 639-43). Tumors formed in these mice histologically and biochemically, were extremely similar to human SCC tumors. We showed that while laminin-5 negative keratinocytes showed no tendency to form tumors after Ras/IKB transformation, retroviral transfer of laminin-5 cDNA restored both expression of laminin-5 and restored the capacity of these cells to form tumors. These results are significant in that they demonstrate that laminin-5 expression is absolutely required for SCC development.

[0185] As an extension of these tumor studies, we next studied the capacity of truncated laminin a3 chain to support SCC development. We Ras/IKB transformed JEB keratinocytes expressing a3 wild type (WT), 1337Tr or LacZ and injected SQ into nude mice. Through two sets of experiments with eight mice per condition, we found that the 1337Tr a3 chain expressing cells did not form any tumors and were most similar to LacZ negative controls (Figure 7). In addition, 1450Tr cells, though one set of experiments and

four mice total per condition, fail to produce SCC tumors either. Despite a lack of tumor formation,1337Tr and 1450Tr cells at injection sites clearly showed expression and extracellular deposition of mutant laminin-5 molecules. These results are significant in that they demonstrate that the G4-5 domain of laminin-5 is essential for SCC development.

Example 2. Cloning of G4 and/or G5 MF sequences

[0186] The laminin α3 chain is processed at residues 1337-1338, according to N-terminal sequencing studies (Tsubota et al., 2000, *Biochem. Biophys. Res. Commun.*, 278: 614-620). As shown in preliminary results, we have produced a human laminin α 3 cDNA (1337Tr) which codes for a protein truncated at amino acid 1337, simulating the cleavage product, and have also produced 1450Tr, an α 3 cDNA truncated at amino acid 1450. We propose to produce another laminin α3 cDNA (1551Tr), truncated near the beginning of the G5 domain at amino acid 1551. PCR primers will be designed to produce a product that spans from nucleotide 2771 to nucleotide 4653 of the full length wild type laminin α3 cDNA. This will include a unique BstII site on the laminin α3 cDNA, which will be on the 5' end of the PCR product, and a NotI site will be engineered into the 3' side of the PCR product. The full length laminin α3 cDNA in pENTR1A© (Invitrogen) Gateway plasmid will be cleaved with BstII and NotI enzymes and the PCR product described above will be ligated into the vector with the 3' end ligating with the BstII site in the laminin a3 cDNA, and the 3' end ligating with the pENTR1A multiple cloning site. This cloning product, which will be confirmed by sequencing, will comprise cDNA coding for the laminin α3 chain amino acids 1-1551. The laminin α3 1551Tr cDNA will then be transferred from the pENTR1A plasmid to a Gateway adapted LSRZ retroviral vector through lambda phage recombination reactions.

[0187] Three cDNA constructs coding for laminin α3 G domain will be produced. One termed G4 will code for amino acids 1338 to 1560, one termed G5 will code for amino acids 1560 to 1713, and a third termed G4-5 will code for amino acids 1338 to 1713. We will produce each by PCR of the wild type laminin α3 cDNA. In one PCR experiment, we

will engineer an EcoR1 tail at either end of each of the three PCR products for cloning into the bacterial expression vector pGEX (Amersham). These constructs will be confirmed by sequencing, and then utilized to produce purified G4, G5 and G4-5 domains in a bacterial expression system.

[0188] In another PCR experiment, we will insert an NheI tail on the 5' side and a Not I tail on the 3' side of each of the G4. G5 and G4/5 PCR products. These will be cloned into the mammalian expression vector pCEP which contains a BM40 signal sequence to which cDNA can be cloned to via an NheI restriction site. We have previously used the BM40 signal sequence in this vector to successfully promote secretion of collagen XVII ectodomain (Areida et al., 2001, J. Biol. Chem., 276: 1594-601). The G4, G5 and G4-5 will each be cloned into pCEP vector, to pick up the BM40 signal sequence, then the BM40 signal sequence and laminin α3 G domain cDNA will be removed from the vector by KpnI and NotI restriction sites and ligated into the pENTR1A Gateway vector, and by lambda phage recombination, the laminin α3 G domain cDNAs with their BM40 signal sequences will be cloned into a Gateway adapted LZRS retroviral plasmid.

Example 3. Assays for Detecting Inhibition of SCC Tumorigenesis

[0189] At present, it is unclear whether G4-5 domain performs its function in SCC before or after it becomes processed and dissociated from laminin-5. We propose to test this question by attempting to restore tumor generating capabilities in Ras/IKB transformed 1337Tr keratinocytes by adding exogenous G4-5 protein or G4-5 cDNA. If Ras/IKB transformed 1337Tr keratinocytes can form tumors in nude mice after receiving G4-5 protein or cDNA, this would indicate that the G4-5 domain is active in SCC tumors in a soluble form.

[0190] Matrigel, which contains heparin sulfate proteoglycan as one of its primary constituents, is the material in which we suspend our Ras/IKB tumors cells in, during subcutaneous injection into nude mice. As the laminin a3 G4-5 domain has heparin binding

properties (Amano et al., 2000, J. Biol. Chem.., 275: 22728-35), we will suspend the G4-5 domain at 100 μg/250μl into Matrigel and use it as a substrate for injection of Ras/IKB transformed 1337Tr keratinocytes. We will use laminin α3 G4-5 domain (as described in Aim 1) suspended in Matrigel as a control. We hypothesize that laminin G4-5 domain will remain localized to the Matrigel impregnated matrix surrounding tumor cells and will be slowly released as matrix is gradually remodeled by tumor cells. We will test this on four mice injected with Ras/IKB treated 1337 cells embedded in G4-5 domain containing Matrigel, using four serial biopsies at 1 week intervals by IDIF using G4-5 domain specific antibodies to assess the persistence of G4-5 domain protein in injection/tumor sites.

[0191] If laminin α3 G4-5 domain protein is detected and shown to persist in injection sites, we will perform a second set of experiments injecting Ras/IKB transformed cells embedded in Matrigel containing either laminin α3 or laminin α3 G4-5 domain. These cells will be injected into nude mice and assessed over the course of 4 weeks for tumor development. Wild type Ras/IKB transformed keratinocytes will be used as a positive control and 6 mice per condition will be used.

[0192] Alternatively, the laminin G4 domain cDNA can be delivered by gene therapy as described below. This technique should promote long term G4-5 domain expression in 1337 Tr cells over the course of the 4 week assay.

[0193] Laminin G4-5 domain cDNA will be cloned into LZRS retroviral vector. LacZ or laminin G4-5 cDNA containing retrovirus will be used to infect 1337Tr keratinocytes. Cells will be selected with Blasticidin, transformed with Ras/IKB and then injected into nude mice. Six mice per condition will be assessed over 4 weeks for tumor growth as previously described (Dajee et al, 2003, Nature, 421: 639). Tumors will be analyzed by IDIF using G4/5 or LacZ antibodies to verify secretion of retroviral cDNA products.

[0194] The effects of laminin α3 G4 and laminin α3 G5 antibodies on tumor development. We will inject sufficient antibody to maintain a circulating titer of 1:1000 as tested by dilution of mouse sera by Western blot analysis of G4-5 domain protein. Laminin

α3 G4, G5 and G4-5 domains cloned into pGEX vector as outlined above will be utilized to produce G4, G5 and G4-5 domain bacterial fusion proteins. Proteins will be affinity purified on a GST column, and GST tags will be subsequently removed by enterokinase (Invitrogen). Isolated G4 and G5 domain proteins will then be used to produce rabbit polyclonal antisera at Josman Labs, Napa, CA, according to their recommended protocols.

[0195] Once high titer polyclonal antisera is obtained, additional G4, G5 and G4-5 protein will be produced, affinity purified and coupled to a Sepharose CL-4B column at a concentration of 0.5 mg protein per ml of gel. G4 polyclonal antisera will be affinity purified on a G4-sepharose column and G5 antisera will be affinity purified on a G5 sepharose column. Affinity purified G4, G5 and G4-5 antibodies will be dialyzed into PBS and filter sterilized. Initially, we will test the antibodies (G4, G5, G4-5) by IP injection of immunodeficient mice bearing human foreskin xenografts by a technique which we have utilized previously (Li et al., 2003, EMBO J., 22:2400-2410). Titers of circulating antibodies in treated mice will be assessed at 3 day intervals using sera obtained from tail vein bleeds. The amount of antibody injected and the injection intervals will be adjusted to maintain a titer sufficient to detect laminin G4-5 protein by Western blot at a 1:1000 serum dilution. We will clinically assess foreskin grafts and mouse skin over the course of three weeks of injections to determine whether epidermal separation is noted, and mice will be examined by autopsy to detect any epithelial sloughing of mucosa or internal organs.

[0196] Once the proper antibody dose and injection intervals are obtained, and if mice are able to tolerate antibody injections, we will go on to perform antibody inhibiton of Ras/IKB wild type keratinocyte derived tumors. In these studies, nude mice will be administered periodic G4, G5 or G4-5 antibody injections to maintain a constant circulating antibody titer as described above. Once antibody titers are initiated, then mice will receive SC injections of Ras/IKB transformed human keratinocytes. Three groups of 6 mice each will be studied, using affinity purified laminin a3 G4 antibody, laminin a3 G5 antibody or mouse IgG. G domain antibody conditions will be analyzed for tumor growth and tumor histology as previously described (Dajee et al., 2003, Nature, 421:639-43).

[0197] While the foregoing has presented specific embodiments, it is to be understood that these embodiments have been presented by way of example only. It is expected that others will perceive and practice variations which, though differing from the foregoing, do not depart form the spirit and scope of the inventions as described and claimed herein. All patent applications, patents, and literature references cited in this specification are hereby incorporated by reference in their entirety. In case of conflict or inconsistency, the present description, including definitions, will control.